1995

Oxidative and frying stabilities of soybean oils with altered fatty acid and/or lipoxygenase contents

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Oxidative and frying stabilities of soybean oils with altered fatty acid and/or lipoxygenase contents

by

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A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Food Science and Human Nutrition
Major: Food Science and Technology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Members of the Committee:

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Iowa State University
Ames, Iowa

1995

To my parents, my wife and my daughter!

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GENERAL INTRODUCTION

The production and widespread use of soybean oil have shown its importance. Soybean oil is very susceptible to oxidation during storage at room temperature and to polymerization during frying at high temperatures mainly because of the high polyunsaturation content of its fatty acids. This instability of soybean oil has been a challenge to food scientists. In addition, the trend towards more healthful, polyunsaturated oils with little off-flavor has made the soybean oil industry face changes to meet consumers' demands.

To overcome its instability for high temperature use, such as for deep-fat frying, soybean oil is usually partially hydrogenated to reduce unsaturation. Hydrogenation of oils, however, results in the formation of some *trans* fatty acids (*t*FA), a topic of recent attention after Mensink and Katan (1990) reported that consuming *t*FA increased low-density lipoprotein cholesterol and decreased high-density lipoprotein in human plasma. The effect of *t*FA may be similar to that of saturated fatty acids after consumption. The saturated fatty acids are believed to be the most effective dietary factors in increasing plasma cholesterol (Dupont et al., 1991). The majority of *t*FA (80%) in the human diet comes from consumption of hydrogenated vegetable oils, with the average per capita intake ranging from 6.5 g/day to 12.0 g/day of *t*FA (Borenstein, 1991). Because of the awareness of *t*FA issue, margarine consumption in Canada has declined at least partly because of the *t*FA issue (Mag, 1994). The U.S. Food and Drug Administration reviewed whether the labels of processed foods should include

information on dietary *t*FAs (Anderson, 1995). Thus, the future of the hydrogenation process is uncertain.

Unsaturation of fatty acids is the key factor in determining oxidative stability of an oil (Gray, 1978; Frankel, 1982). Lipoxygenase, however, also contributes to off-flavor development in soy foods (Davies and Nielsen; 1986, Davies, et al.; 1987, Frankel et al., 1988; White, 1992; Moreira, et al., 1993; and Kitamura, 1995). For example, soy milk without lipoxygenase-2 had less off-flavor than soy milk from traditional beans (Davies et al., 1987). Kitamura (1995) reported that soymilk without lipoxygenase-2 and -3 and without all three lipoxygenase enzymes had low peaks of volatile compounds recorded on gas chromatograms. The oxidative stability of soybean oil from beans lacking lipoxygenase-1 was not different from that of traditional soybean oil (Frankel et al., 1988). There have been no reports about the influence of lipoxygenase-2 and -3 on the oxidative stability of soybean oil.

It would be beneficial to have an alternative to hydrogenation. The newly developed soybean oil with high 16:0 at Iowa State University (Fehr et al., 1991) may potentially meet consumers' requirement for a non-hydrogenated cooking oil that would avoid *t*FA. Therefore, soybean oil with high 16:0 should be investigated to determine the possibility of replacing hydrogenation. Because of lower price and wide availability of soybean oils, this soybean oil should compete very well with cottonseed oil or palm oil if it shows the better oxidative stability than traditional soybean oil for frying use. The objectives of this research were: (1) to determine the influence of both lipoxygenase enzyme content and fatty acid patterns on the oxidative stabilities of soybean oils at accelerated room temperature and on polymerization at frying temperature; (2) to

determine the influence of high palmitate content on the oxidative stability of soybean oils; and (3) to study the flavor profiles of these fresh and oxidized soybean oils.

Dissertation organization

This dissertation consists of three papers that will be submitted to the Journal of the American Oil Chemists' Society for publication. All papers have been prepared according to the format described by the journal. The three papers are preceded by a general introduction and a literature review, and followed by a general conclusion. Literature cited in the general introduction and literature review is listed in alphabetical order according to author's name, and follows the general conclusion.

LITERATURE REVIEW

Soybean, *Glycine Max.* (L.) Merr., is one of the oldest crops utilized by humans. According to written records, soybeans were cultivated by the Chinese as far back as 2800 B.C. (Watanabe and Kishi, 1984), a fact helpful in understanding why soybeans have been important food crops in Asian countries. Soybeans are mainly consumed in these countries as foods, such as tofu, miso, and soy sauce. The nutritional value of soybeans is probably the reason that soybeans have been used so widely in Asian countries.

Development of the soybean industry in the United States

The history of soybeans in the United States is quite different from that in China. The records show that soybeans were first planted in the United State by Henry Yonge, the Surveyor-General of Georgia, for Samuel Bowen in 1765 (Hymowitz and Harlan, 1983). The soybean, however, was not widely planted in this country until the turn of this century. Even in 1924, soybean production in the United States was only about 0.1 million metric tons (Table 1), from which only one-third of the crop was harvested as grain. The majority was harvested for forage (Dutton, 1981).

The shortage of fats and oils during World War I stimulated the development of the soybean industry. Subsequent improvements in equipment for harvesting,

Table 1. Soybean production in the U.S. and in the world

Year	U.S. production $x10^3$ metric tons	World production $x10^3$ metric tons	U.S. % of total world production
1924	135	-	-
1930	367	-	-
1940	2,177	-	-
1950	7,828	17,222	46
1960	15,239	26,641	57
1970	30,911	41,764	74
1980	49,453	81,774	61
1990	52,302	103,619	51
1994	68,000[a]		

Source: U.S. Department of Agriculture (1924-1991);
[a]Anderson (1995).

processing, and refining made U.S. soybean and soybean products competitive in the world (Horan, 1974). Soybean oil was first processed in the United States in 1911 from beans imported from Manchuria, China (Smouse, 1985). With the increase of soybean planting, the soybean oil industry also was founded. The first hydraulic pressing plant began operation in the U.S. in 1911 to produce soybean oil. The earliest expeller plants were established in 1922. The solvent extractor was put in use between 1923 to 1925. It was Henry Ford who promoted the production of soybean. In 1930, the Ford Motor Company owned three soybean processing plants in Michigan and soybean oil was used in automobile paint and plastic fittings (Smouse, 1985). By 1945, soybean oil production in the United States was 0.59 million tons and exceeded cottonseed oil production for the first time. Since then, soybean oil remained the most widely used edible oil in the United States (Dutton, 1981). Soybean oil generally made up about 75

% of U.S. vegetable oil production in 1994 (Anderson, 1995) and accounted for 40 % of the world vegetable oil production in 1990 (USDA, 1991).

The oxidative stability of soybean oil

Soybean oil contains high amounts of linoleic acid (18:2) and linolenic acid (18:3) (Table 2) compared with other vegetable oils, which makes soybean oil susceptible to autoxidation, resulting in poor quality. To deal with the quality problems of soybean oil, researchers and industrial groups worked together under the organization of the Board of the National Soybean Processors Association. Extensive research on the mechanisms of oil oxidation was conducted.

Dutton et al. (1951) performed a classic experiment in which 9% 18:3 was interesterified into the glyceride structure of cottonseed oil and the typical reversion flavor of soybean oil was identified. During the same year, Evans et al. (1951) confirmed that trace metals, such as iron and copper, were detrimental to the stability and quality of soybean oil. Thus, stainless steel has been used to replace the other metals used in equipment in the soybean industry, and metal deactivators, such as citric acid, are commonly used to chelate metal ions remaining in the oil (Dutton, 1981).

Since the initial discovery of the role of 18:3 in flavor stability, the mechanisms of autoxidation of lipids have been uncovered. It is known that autoxidation can be

Table 2. Fatty acid compositions (%) of commonly used vegetable oils

Oils	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Corn oil	12.2	2.2	27.5	57.0	0.9
Cottonseed oil	24.7	2.3	17.6	53.3	0.3
Olive oil	13.7	2.5	71.1	10.0	0.6
Palm oil	45.1	4.7	38.8	9.4	0.3
Peanut oil	11.6	3.1	46.5	31.4	0.0
Conala oil	3.9	1.9	64.1	18.7	9.2
Sesame seed oil	9.9	5.2	41.2	43.2	0.2
Soybean oil	11.0	4.0	23.4	53.2	7.8
Sunflower oil	6.8	4.7	18.6	68.2	0.5

Source: White (1992)

initiated by pyrolysis, hydroperoxide decomposition, metal catalysis, and photolysis (Frankel, 1980 a), resulting in free radicals that leads to the formation of hydroperoxides. Hydroperoxides are not stable and readily form decomposition products and polymers. The decomposition products, including free fatty acids, carbonyls, and fatty alcohols, contribute to off-flavor (Gray, 1985; Yoon et al., 1988). The polymers cause dark colors, increase viscosity, and decrease the sensory qualities of fried foods. The polymers also cause foaming and lower the smoke point of frying oils. The destruction of nutrients, such as essential fatty acids and vitamins, and the formation of potential toxic materials may occur during the frying process in the presence of air (Kummerow, 1962; Matsuo, 1962).

To overcome the flavor instability of soybean oil, some practices are currently in use, such as adding metal deactivators and hydrogenating to reduce polyunsaturation. A more recent approach to reducing lipid oxidation is to modify the fatty acid composition of soybean oil through plant breeding. Considerable emphasis has focused

on breeding soybeans with low 18:3 content. Hammond and Fehr (1984) and Graef et al. (1988) reported the development of new soybeans with oils having reduced 18:3. Researchers have found that low-18:3 (3.8%) and high-stearic acids (18:0, 28.1%) soybean oils were more stable than normal soybean oils in room and frying temperature stability tests (White and Miller, 1988; Miller and White, 1988 a, b). Since then, soybean lines with lower 18:3 contents (2%) have been produced (Graef, et al., 1988), resulting in even greater stability (Liu and White, 1992a, b). The newer soybean oils with low linolenic acid (<3%) contents have been released for commercial use (Anonymous, 1994).

In the soybean oil industry, the commonly used way to increase oxidative stability is partial hydrogenation, which introduces the formation of *trans*-fatty acids (*t*FAs). The *t*FAs have physical properties similar to those of saturated fatty acids (Gurr, 1983, Dupont et al., 1991) and higher melting points than other fatty acids with the same carbon number and *cis* double bonds (Sonntag, 1979). Since the *trans* double bond forms nearly linear carbon chain compared with the kinked chain of the *cis* bonds, *t*FA molecules can be packed together in a crystalline array, like the saturated fatty acids, and the melting points of *t*FAs are higher than the corresponding *cis* fatty acids (Gurr, 1983).

In 1990, scientists from the Netherlands (Mensink and Katan, 1990) reported that consuming *t*FA increased low-density lipoprotein cholesterol and decreased high-density lipoprotein in human plasma, which enhanced concerns about hydrogenation of vegetable oil. Even though the evidence for these results has been questioned, people still worry about the effects of *t*FAs. Gurr (1983) proposed the metabolism of *t*FA,

which probably explained the effect of *t*FA on human health. After consumption, fatty acids are transported from the intestine, largely as triacylglycerols in chylomicrons. After absorption, a certain fraction is incorporated into intestinal very-low-density lipoproteins (VLDL) as cholesterol esters. In rats, a larger fraction of *trans*-9-octadecenoic acid (elaidic acid) than oleic acid is transported by being incorporated into intestinal VLDL. The cholesterol esters of elaidic acid and the *trans*-isomers of linoleic acid are hydrolyzed more slowly in tissues than are the *cis*-isomers but faster than saturated acids. The catabolisms of both saturated and unsaturated (*cis*- or *trans*-) fatty acids are very similar (Gurr, 1983).

The intake of *t*FAs in the U.S. diet has been estimated to be from 8.1 g/capita•day (Hunter and Applewhite, 1991) to 13.3 g/capita•day (Enig et al., 1990). Enig et al. (1990) reported that 91.3% of *t*FAs were from vegetable oils. Gurr (1983) believed the average intake was 12 g/capita•day, of which 95.2% was from partially hydrogenated vegetable oils. Because soybean oil accounts for about 75% of all visible oil consumption in the United States, it is appropriate to conclude that partially hydrogenated soybean oil is a main source of *t*FAs in U.S. diet. Therefore, decreasing the intake of partially hydrogenated soybean oil can lower the intake of *t*FA.

Mag (1994) noted that margarine consumption in Canada has declined, at least partly because of the *t*FA issue. The U.S. Food and Drug Administration (FDA) was indicated that it might consider labeling of *t*FA in foods in the future because of data suggesting that *t*FA might raise LDL cholesterol (Haumann, 1994). A recently published report indicated that the FDA was undergoing a review to determine

whether the labels of processed foods should include information on dietary *t*FAs (Anderson, 1995).

Without the hydrogenation procedure, the easiest way to enhance the oxidative stability of soybean oil is to increase saturated fatty acid contents and/or decrease the polyunsaturated fatty acid contents by breeding. It may be beneficial to develop an oil with relatively high saturated fatty acid contents that can be used directly to make shortenings, margarines or stable frying oils without hydrogenation. Researchers at Iowa State University have developed soybean lines containing high palmitic acid (16:0, 23-25%) with different 18:3 contents (from 2.7% to 9.3%). The increased saturated fatty acid content should increase the stability of soybean oil. Naturally saturated oils also would have fewer processing costs, and may result in more profit for the farmers and/or in less cost for consumers. Miller and White (1988 a, b) reported that oil from a soybean line, A6, containing 20% stearate was more stable than oil from commercial soybean varieties with 4%.

There is enough evidence to confirm that the stability of soybean oil is highly dependent upon its fatty acid composition; however, lipoxygenase enzymes also contribute to the flavor instability of oils based on speculation (Davies and Nielsen, 1986; Davies, et al., 1987; Frankel et al., 1988; Endo, et al., 1990; White, 1992; Moreira et al., 1993; Kitamura, 1995). Lipoxygenases are a group of enzymes that catalyze hydroperoxidation by molecular oxygen of methylene interrupted *cis*-, *cis*-1,4 pentadienes (Andrawis et al., 1982). In soybean oils, lipoxygenases can oxidize 18:2 and 18:3 rapidly to form off-flavors. Lipoxygenase occurs in multiple forms, which are often referred to as lipoxygenase-1 (LOX 1), lipoxygenase-2 (LOX 2), and lipoxygenase-3

(LOX 3), (Chism, 1985). Each isozyme has different substrate specificity, causing differences among the isozymes in how they affect soy food. The role of lipoxygenase on soybean oil stability has been studied in only a limited combination. LOX 1 was reported not to be a factor in determining the oxidative and flavor stability of soybean oil (Frankel et al., 1988; Endo et al., 1990).

Although a fairly clear picture of the mechanisms of lipid oxidation has been elucidated, work is still needed to understand the mechanisms of oxidation that will help to monitor and control oxidation. Lipid oxidation is usually classified into three categories, hydrolytic rancidity (hydrolysis), oxidative rancidity, and flavor reversion. Hydrolytic rancidity is not a wide-spread problem in the food industry, but is a great problem in dairy foods. Oxidative rancidity and flavor reversion are of greatest concern to the soybean oil industry.

Hydrolytic rancidity

Hydrolytic rancidity results from the lipolysis of triacylglyceride (TAG), in which fatty acids are cleaved, either by lipase or by chemicals, from the glyceride backbone to form free fatty acids (FFA). If the FFAs are short-chain, such as in milk fat, they are odorous and contribute to the rancid flavor in fats (Hammond, 1989). Hydrolytic rancidity is different from oxidative rancidity in that it does not need oxygen for the reaction, but is accelerated by the presence of moisture, high temperature, and lipases (Potter, 1986).

Hydrolytic rancidity generally is a major problem in the dairy industry. Rancid flavors in milk and some other fluid products are undesirable. On the other hand, the flavors resulting from hydrolytic cleavage of fats in certain dairy products, such as cheese, are favorable (Weihrauch, 1988). The most widely used method to monitor lipolysis in milk is acid degree value (ADV). Normal raw milk usually has an ADV of 0.25 to 0.40 mEq/L. If the ADV goes to 1.2, the rancid flavor is obnoxious (Bianco et al., 1978). The sensory quality interpretation of ADV is listed in Table 3.

In the food industry, lipolysis of vegetable oils and other fats mainly occurs during deep-fat frying as a result of large amounts of water from the food and the high temperature. The increase in FFA during frying is accompanied by a decrease of the smoke point, surface tension of the oil, and the quality of the fried food. The released FFAs are more readily oxidized than are fatty acids esterified to glycerol; therefore, the FFAs shorten the usable life of the oil (Nawar, 1985 b).

Table 3. Sensory quality interpretation of ADVs (the amount of 1 N KOH required to titrate 100 g of fat) in milk fats

ADV (mEq/L)	Interpretation
<0.4	Normal
0.7 to 1.1	Borderline (indefinite)
1.2	Slight rancidity
1.5 and above	Unsatisfactory (extreme hydrolytic rancidity)

Source: Bianco et al. (1978).

Oxidative rancidity

Oxidative rancidity is common to all unsaturated fats and oils. There are two major categories of rancidity, autoxidation and photooxidation, both of which lead to the formation of hydroperoxides. Decomposition of hydroperoxides and origination of volatiles, however, are not completely known.

Autoxidation

Autoxidation is a series of free-radical chain reactions, in which unsaturated fatty acids react with oxygen to form hydroperoxides (Frankel, 1980 a). The hydroperoxides also can break down to form secondary oxidative products that include volatile compounds and polymers.

Free radical mechanism The most accepted mechanism for the autoxidation of unsaturated fatty acids (RH) is the free radical reaction, which usually includes three steps: initiation, propagation, and termination. The term, free radical, refers to any atom or group of atoms that has one or more unpaired electrons. A free radical, having no positive or negative charge, is highly reactive because of its unpaired electron and is considered to be a high-energy, highly reactive, short-lived, and nonisolable reaction intermediate (Fessenden and Fessenden, 1986). These reactions can be illustrated as follows (Table 4). Ks are empirical constants.

Table 4. Free radical reactions

Reactions	Illustration of reactions in each step
Initiation:	Initiator $\xrightarrow{k_1}$ free radicals ($R^{\cdot}$, $ROO^{\cdot}$)
Propagation:	$R^{\cdot} + O_2 \xrightarrow{k_2} ROO^{\cdot}$
	$ROO^{\cdot} + RH \xrightarrow{k_3} ROOH + R^{\cdot}$
Termination:	$R^{\cdot} + R^{\cdot} \xrightarrow{k_4}$
	$ROO^{\cdot} + R^{\cdot} \xrightarrow{k_5}$ } Nonradical products
	$ROO^{\cdot} + ROO^{\cdot} \xrightarrow{k_6}$

Source: Nawar (1985 a).

The rate of oxygen absorption can be expressed as

$$\text{Rate} = -d[O_2]/dt = \{K_a[RH][ROOH]\}/\{1 + \lambda[RH]/p\}$$

RH is the substrate fatty acid (H is an α-methylenic hydrogen atom easily detachable because of the activating influence of the neighboring double bond or bonds), ROOH is the hydroperoxide formed, p is the pressure of oxygen, and λ and K_a are empirical constants.

At high oxygen pressure (λ[RH]/p is much smaller than 1), the above equation can be simplified to:

$$\text{Rate} = k_3(k_1/k_6)^{1/2}[ROOH][RH]$$

Thus, the rate of oxygen absorption is independent of oxygen pressure. At low oxygen pressure (λ[RH]/p is greater than 1), the equation can be rewritten as:

$$\text{Rate} = k_2(k_1/k_4)^{1/2}[\text{ROOH}][O_2]$$

Initiation, the first step toward off-flavor development, must be catalyzed by thermal dissociation, photodecomposition of hydroperoxides, metal catalysis, or ultraviolet irradiation (Frankel, 1985) because the reaction (RH + O· ⟶ free radicals) is thermodynamically difficult (activation energy of about 35 kcal/mol) (Nawar, 1985 a). That is why oil products should be stored in the dark at low temperature to avoid contacting with metal. The degree of unsaturation in fatty acids also influences the formation rate of free radicals because the more unsaturation, the more unstable the oil (Gray, 1978). Gunstone and Hilditch (1945) reported that the relative rates of autoxidation of methyl oleate, linoleate, and linolenate at 20 °C was 1:12:25.

During propagation reactions, free radicals react with oxygen to form hydroperoxides, which can be decomposed to form various volatiles, such as aldehydes, alcohols, acids, and new free radicals (Frankel, 1982). There are several pathways in this stage, and each pathway produces different volatile compounds. Figure 1 shows several pathways possibly occurring during the decomposition of hydroperoxides (Lea, 1962).

In termination reactions, nonradical products are formed. Hydroxylic and ketonic compounds and oxygen are formed during this step (Russell, 1957). Therefore, even at termination, some volatile compounds may be formed.

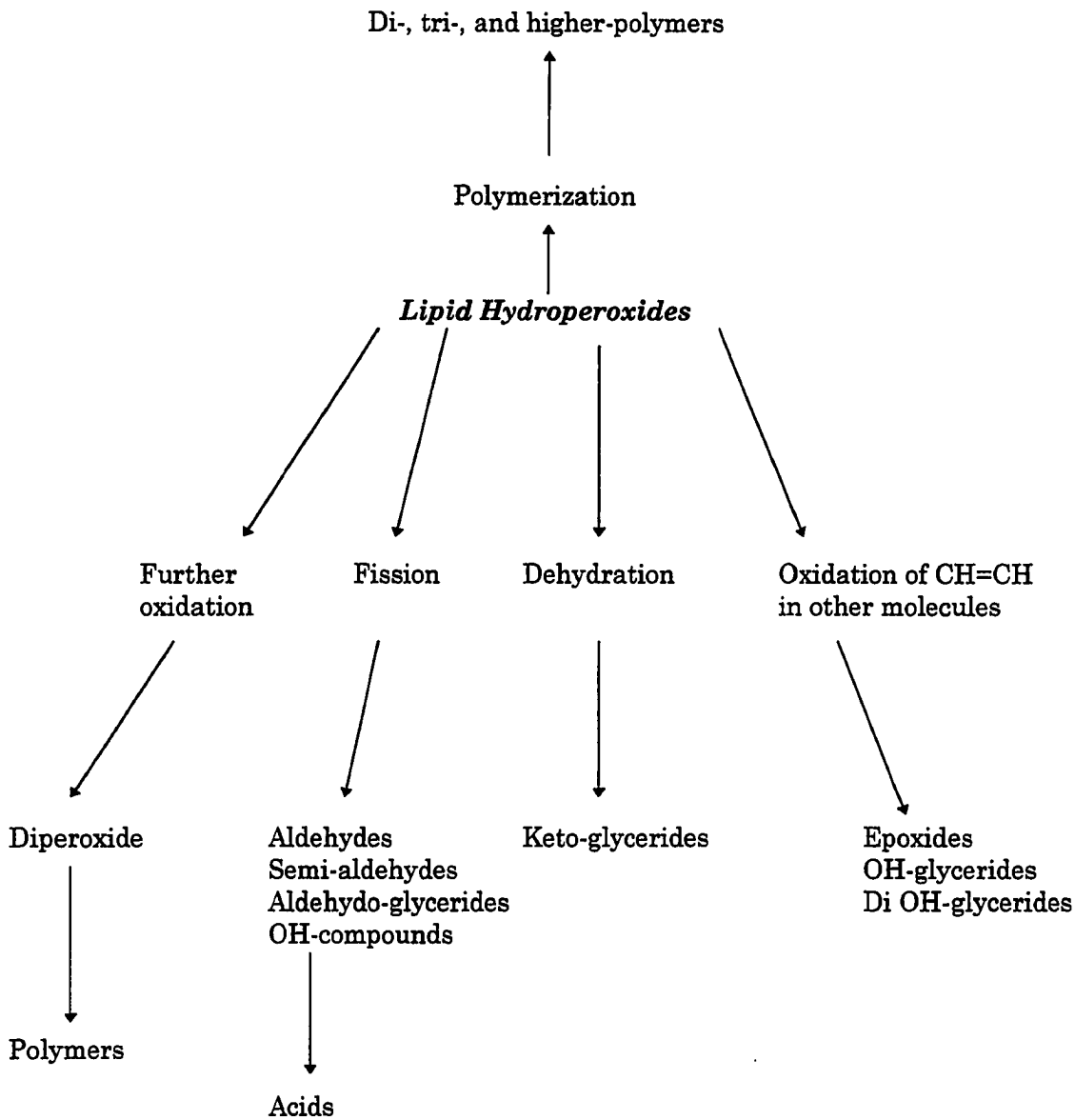

Figure 1. Possible pathways for the decomposition or polymerization of hydroperoxides (Lea, 1962)

If chain-breaking antioxidants (AH) are added, the chain reactions will be terminated, as illustrated below (Frankel, 1980 a), thus retarding all oxidation reactions. The commonly used synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and butylated hydroquinone (TBHQ) and have been approved for use in edible fats and oils (Table 5).

Another group of antioxidants is known to be preventive antioxidants and include metal deactivates, peroxide destroyers, and ultraviolet light deactivators. Metal deactivators chelate metal ions that stabilize edible fats or oils, and include citric, phosphoric and ascorbic acids. Peroxide destroyers react with hydroperoxides to give stable products by nonradical processes and include sulfur compounds, phosphites, and phosphines. These compounds reduce hydroperoxides into the more stable alcohols that are not approved for use in foods. Ultraviolet light deactivators absorb irradiation without the formation of radicals and are pigments such as carbon black, phenyl salicylate, and α-hydroxy-benzophenone. There is no information about them for food use (Frankel, 1980 a).

Table 5. Reactions involving antioxidants

Reactions		Products
$ROO^{\cdot} + AH$	$\longrightarrow$	$ROOH + A^{\cdot}$
$ROO^{\cdot} + A^{\cdot}$	$\longrightarrow$	Nonradical products
$A^{\cdot} + A^{\cdot}$	$\longrightarrow$	Nonradical products

Source: Sherwin (1978)

Synergism often is observed, which is the reinforcing effect of a system with several added stabilizers exhibiting a greater combined effect than the sum of their individual effects. Significant synergism generally is obtained when chain-breaking and preventive antioxidants are used together because they suppress both initiation and propagation (Frankel, 1980 a). The most common synergistic effect in foods is the use of antioxidants with metal deactivators.

Factors affecting autoxidation The most important factor affecting autoxidation is degree of unsaturation of the oil. There are other factors that also can initiate or accelerate the autoxidation. Some major factors will be briefly discussed here.

Temperature Temperature has a critical effect on lipid oxidation and should be kept as low as possible during processing and storage of the oil. Between 20 and 60°C, every 15°C increase in temperature doubles the speed of oxidation (Johansson, 1976). It is recommended that an oil be stored only 5-10 °C above its melting point (Johansson, 1976).

Oxygen concentration At very low oxygen pressure, the oxidation rate is directly related to oxygen pressure. The influence of oxygen is also associated with temperature and surface area of the oil (Nawar, 1985 a). When oils are stored, they should be placed in a sealed container covered with nitrogen to minimize the oxygen dissolved in the oils. When conducting a storage test, the ratio of surface area to the volume of oil should be taken in account to minimize the influence of oxygen.

Off-flavor at low temperature Hydroperoxides, which are formed during propagation, are very unstable and readily produce decomposition compounds. Different hydroperoxides will be formed from different fatty acids and, therefore, different decomposition compounds will result. Frankel (1991) reported that linolenate can be oxidized to form monohydroperoxides, hydroperoxy epidioxides, and a small amount of dihydroperoxides. If trilinolenate fatty ester is used as a model, a mixture of eight isomers of *cis, trans-* and *trans, trans*-conjugated diene-triene 9-, 12-, 13- and 16-hydroperoxides are formed through free radical autoxidation. If photosensitized oxidation is involved, *cis, trans*-unconjugated triene 10- and 15-hydroperoxides also are produced (Frankel, 1982). This variety of decomposition products makes the final volatile compounds even more complicated when considering that other unsaturated fatty acids are involved.

Off-flavor at high temperature Deep-fat frying is a method that is commonly used to manufacture and prepare foods throughout the world (Paulose and Chang, 1973). Vegetable oils, especially partially hydrogenated soybean oil, are often used as deep-fat frying oils. At frying temperature, oxidation is even more complicated, resulting in a series of changes and reactions to form numerous decomposition products, as illustrated in Figure 2 (Fritsch, 1981). As these reactions continue, the functional, sensory and nutritional quality of frying oils can be changed to a point where high quality foods can no longer be prepared (Fritsch, 1981).

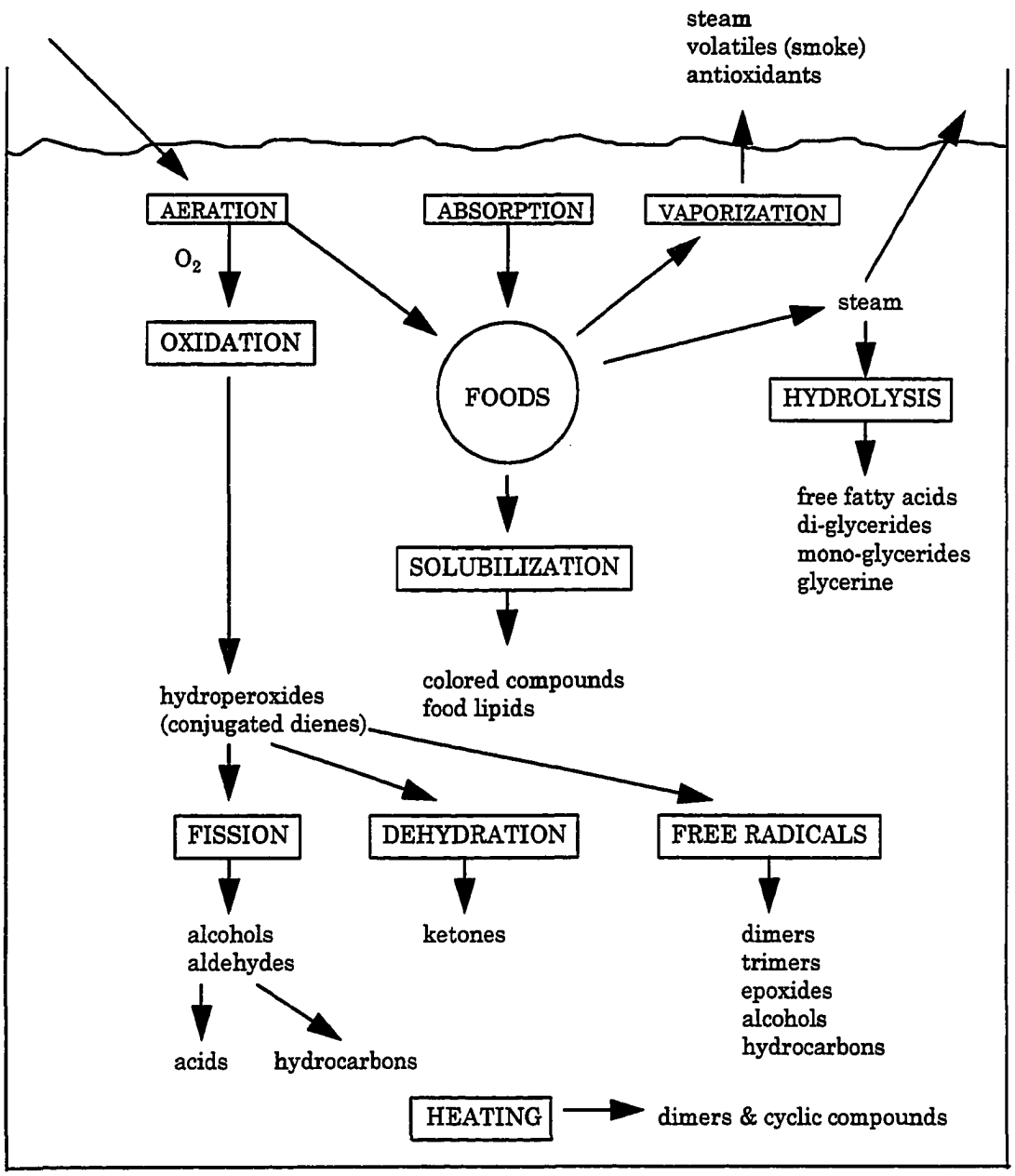

Figure 2. Reactions and changes occurring during deep-fat frying (Fritsch, 1981)

During deep-fat frying, the oil is used at high temperatures in the presence of air. Under such conditions, both thermal and oxidative decomposition of the oil can occur, resulting in the formation of both volatile and nonvolatile decomposition products. (Chang et al., 1978). The nonvolatile decomposition compounds cause foaming when foods are fried in the oil. The foaming is often regarded by food processors as an indicator that the frying oil should be replaced. Foods fried in such oils may contain a sufficient amount of decomposition products to have adverse effects to the safety, flavor, flavor stability, color, and texture of the fried foods (Chang et al., 1978). Melton et al. (1994) believed that the nonvolatile decomposition products are predominantly generated from oxidation reactions, but may also be formed from the thermal or pyrolytic pathway.

To minimize the oxidation of oils in deep-fat frying, antioxidants are usually added to the oils. Soybean oils containing low contents of 18:3 and high contents of saturated fatty acids have been tested for their oxidative stability. Miller and White (1988 b) reported that soybean oils with low 18:3 (A5) and high 18:0 (A6) contents were more stable than those from commercial varieties when held at high temperature. The formations of conjugated dienoic acids from A5 and A6 were significantly less than those from commercial oil. Liu and White (1992b) also found that soybean oils with low 18:3 content were more stable than canola oil and oil from the commercial soybean varieties, Hardin and BSR 101.

There are many papers dealing with the oxidation of oils at frying temperature. Many researchers have tried to elucidate the mechanisms of oxidation under these conditions. Saturated fatty acids generally are more stable than unsaturated ones.

Nawar (1985 b) stated that the saturated lipids also can be oxidized if they are heated in the air at temperatures greater than 150 °C. The predominant oxidative products include a series of carboxylic acids, 2-alkanones, n-alkanals, lactones, n-alkanes, and 1-alkenes.

The unsaturated lipids are easily oxidized. Regardless of temperature, the main reaction pathways of lipid oxidation are basically the same, involving the formation and decomposition of hydroperoxide intermediates, and are predictable according to the location of the double bonds (Nawar, 1985 b). Peroxides, however, decompose rapidly at high temperature. Lomanno and Nawar (1982) demonstrated that when ethyl linolenate was heated at 250 °C, the peroxide values were 44 after 4 min of heating, 198 after 10 min of heating, and zero after 30 min of heating, which also indicated the high speed of hydroperoxide decomposition. In another study, the major volatile decomposition products from heated-oxidized linolenate and isomeric hydroperoxides included acrolein, propanal, 3-hexenal, 2,4-heptadienal, and 2,4,7-decatrienal (Frankel, 1982). Besides the volatile compounds produced during thermal processing, the formations of oxydimers or polymers at elevated temperature in air also occur (Nawar, 1985 a).

The volatile fraction is only a very small portion of the total products of thermal oxidation of unsaturated fatty acids. For example, when one gram of ethyl linoleate is oxidized in air at 180 °C for one hour, nearly 50% of the substrate is changed into other products, of which the total volatiles amount is only 1%. The major decomposition products are non-volatile, which are mainly dimeric and polymeric compounds. In the

presence of oxygen, oxydimers or polymers contain hydroperoxide, hydroxide, epoxide and carbonyl groups, as well as ether and peroxide bridges (Nawar, 1985 b).

Photooxidation

Another way to form hydroperoxides without free radical mechanisms is via photosensitized oxidation, in which oxidation is sensitized by chromophoric impurities in the oil, such as chlorophyll, pheophytin, myoglobin, and porphyrins (Sonntag, 1979). Oxygen is excited into the singlet state through the energy transfer mechanism from a triplet sensitizer that has been exposed to light (Foote, 1968). The singlet oxygen reacts with methyl linoleate at least 1500 times faster than normal oxygen to form hydroperoxides. The decomposition of hydroperoxides produced by singlet oxygen may result in the initiation of normal free-radical autoxidation (Frankel, 1991).

When electrons are charged, they, like magnets, exist in two different orientations, but with equal magnitude of spin, +1 and -1. The total angular momentum of the electrons in an atom is expressed as $2S + 1$ where S is total spin. If oxygen has two unpaired electrons in its outer orbitals, they can align their spins parallel or antiparallel with respect to each other, giving two different states, $2(1/2 + 1/2) + 1 = 3$ and $2(1/2 - 1/2) + 1 = 1$. They are called the triplet ($^3O_2$) and singlet states ($^1O_2$), respectively. In the triplet state, the two electrons in the antibonding $2p\pi$ orbitals have the same spin but are in different orbitals. These electrons, apart via Pauli exclusion, have only a small repulsive electrostatic energy. In the singlet state (an excited state), electrostatic repulsion is great because the two electrons have

opposite spins (Nawar, 1985 a). The characteristics of oxygen at different states are listed in Table 6.

There are two different pathways for photosensitized oxidation of unsaturated fats. Type I is a sensitization of a substrate-sensitizer, such as linolenate-riboflavin, and a formation of diene-type radicals to yield the same type of hydroperoxides as does autoxidation. It involves association of the triplet sensitizer with the acceptor to react with ground-state triplet oxygen to form hydroperoxides. Type II is a reaction in which molecular oxygen is involved to react with the sensitizer after light absorption to form different hydroperoxides. The singlet sensitizer transfers energy to ground-state triplet oxygen to yield singlet oxygen, which reacts with an acceptor to form hydroperoxides (Frankel, 1985 and Sonntag, 1979). The mixtures of isomers resulting from Type II were different from those obtained by autoxidation (Chan, 1977).

Table 6. The energy of oxygen at different state

State of the oxygen molecule	Occupancy of highest orbitals	Energy above ground state
Second excited state ($^1\Sigma_g^+$)	↑ ↓	37 kcal
First excited state ($^1\Delta_g$)	↑↓ —	22 kacl
Ground state ($^3\Sigma_g^-$)	↑ ↑	—

Source: Foote, 1968.

Oxidation of saturated fatty acids

Saturated fatty acids are relatively stable at room temperature. The very high temperature (180-250 °C) during deep frying, however, can decompose saturated fatty acids to form substantial non-oxidative products. When oil is heated in air at temperatures higher than 150 °C, saturated fatty acids can be oxidized to produce carboxylic acids, 2-alkanones, n-alkanals, lactones, n-alkanes, and 1-alkenes (Nawar, 1985 b).

Flavor reversion

A specific problem of soybean oil is the potential development of reversion flavor, which has been described as a return to a previous flavor condition. The off-flavor developed during reversion, however, may not be identical with the original flavor of the crude oil, thus, "reversion" is a term that is often misused (Frankel, 1980 b). Smouse (1985) suggested using the term retrogression, a process of retrograding from a better to a worse condition, to precisely describe reversion. The term reversion, however, is still commonly used simply because people are accustomed to doing so.

Characteristics of reversion flavor

Reversion occurs not only in soybean oil, but also in other oils. Actually, no oil is totally free of reversion tendencies. The only difference is that some oils revert at faster rates than other oils (Smouse, 1985). Unfortunately, soybean oil readily develops a reversion flavor, which is quite undesirable.

Flavor reversion develops at low levels of oxidation, with peroxide values usually below 10 (Frankel, 1980 b). During flavor reversion, the flavor of a vegetable oil is changed from bland to beany, grassy and finally to painty or fishy (Sonntag, 1979). If a soybean oil is stored beyond the reversion, the peroxide value will increase dramatically and the off-flavor will be changed from that of reversion flavor to oxidized flavor (Ullrich and Grosch, 1988).

According to the description given above, a basic question is proposed: What reaction is responsible for flavor reversion? If it is oxidation, the antioxidants should retard the off-flavor development, but they do not. If it is not oxidation, there is some evidence to oppose it, such as the acceleration by prooxidant metal. Many researchers believe that flavor reversion involves some oxidation (Frankel, 1980 b).

Hypothetical mechanism of flavor reversion

To elucidate the mechanism of flavor reversion, numerous papers have been published in an attempt to identify its components and determine the precursors of reversion. Several hypotheses have been proposed trying to explain the mechanism of

reversion. None of these theories, however, is universally accepted. Even today, there are still papers appearing which support or contradict certain theories of flavor reversion (Guth and Grosch, 1990 a, b; Endo et al., 1991).

Linolenic acid theory Linolenic acid is the most important precursor of the reversion flavor compounds (Durkee, 1936; Dutton et al., 1951; Smouse, 1985). In 1951, Dutton et al. conducted a classic experiment with two approaches for determining the precursors for reversion flavor. The first approach was to lower the 18:3 content to 2 to 3% in soybean oil by furfural extraction (Schwab et al., 1950). Fractionating of soybean oil by means of liquid-liquid techniques with furfural was used. When crude soybean oil was contacted with furfural below the temperature of miscibility, two fractions were obtained. The raffinate or oil predominant phase was more concentrated in saturated glycerides and the extract contained the more unsaturated glycerides, free fatty acids, chlorophyll, other pigments, and unsaponifiable constituents (Gloyer, 1949). The raffinate fraction of the soybean oil was less likely to develop reversion flavors than was unfractionated soybean oil. The second approach was to interesterify 9% 18:3 into cottonseed oil. After storage, the cottonseed oil produced the same flavor as reverted soybean oil as judged by a sensory panel. It is, therefore, reasonable to consider linolenic acid a precursor of reversion flavor.

Since then, several papers have supported the theory of the involvement of linolenic acid in reversion of soybean oil (Hoffmann, 1961 a, b; Smouse and Chang, 1967; Chang et al., 1983). Some compounds have been identified and are believed to have 18:3 as their precursor. The compounds include acetaldehyde, propanal, 2-

pentenal, 3-*cis*-hexenal, 3-*trans*-hexenal, *trans, trans* 2,4-heptadienals, *trans, cis* 2,4-heptadienals, and 2 (*cis* and *trans* 1-pentenyl) furans (Smouse, 1985), 1,5-*cis*-octadien-3-one, and *trans, cis* 2,6-nonadienal (Ullrich and Grosch, 1988).

Some data, however, contradict the linolenic acid theory. Mounts et al. (1981) studied the effect of TBHQ on the storage stability of soybean oil and found that the flavor stability of soybean oil, regardless of hydrogenation, was not improved by the addition of TBHQ, which is a very effective antioxidant in retarding oxidation of fats and oils. In their experiment, three soybean oils were used; one was unhydrogenated (iodine value = 137.7, % 18:3 = 8.3), one was hydrogenated with a nickel catalyst (iodine value = 109.1, % 18:3 = 3.3), and one was hydrogenated with a copper-chromium catalyst (iodine value = 112.8, % 18:3 = 0.4). Hydroperoxide development was sufficiently reduced in oils treated with TBHQ during a storage test. The flavor stability of the three oils, measured by sensory evaluation, however, was not increased by treatment with TBHQ. Therefore, the development of off-flavor was likely from fatty acids other than 18:3. These data suggest that 18:3 is likely not the only cause of the reversion flavor in soybean oil.

Isolinoleic acid theory Isomers of linoleic acids are called isolinoleic acid. During hydrogenation, many reactions of the oils can occur at various rates. When 18:3 is hydrogenated, some of the products may be isolinoleic acids (Smouse, 1985). Isolinoleic acid is unreactive and accumulates in hydrogenated products because it has two isolated double bonds that cannot be conjugated (Frankel, 1980 a). Isolinoleic acid

formed during hydrogenation may contain a terminal pentene radical, which is believed to be related to flavor reversion (Frankel, 1980 b).

Frankel (1980 a) believed the oxidation of isolinoleate could produce hydroperoxides by allylic attack of oxygen on each double bond. He used a 9,15-diene fatty acid as a model to illustrate the formation of volatile compounds. After oxygen was added to carbon-15, the hydroperoxide decomposed to form a mixture of aldehydes, similar to those formed from linolenate. Many of those compounds have been found in oxidized soybean oil (Frankel, 1980 a). Therefore, isolinoleic acid may be associated with the development of reversion flavor.

Oxidative polymer theory When 18:3 is oxidized, it polymerizes quickly into a complex mixture containing dimers, trimers, higher polymers, and oxygen (Frankel, 1980 b). These oxidative polymers contain oxygen through carbon to oxygen bonds (Chang and Kummerow, 1953 a). Chang and Kummerow (1954) studied polymers formed during the autoxidation of soybean oil at 60 °C. After fractionation and characterization, they identified several compounds that also were found in reverted soybean oil. They concluded that the polymers may serve as precursors of reversion compounds. Their conclusion was also supported by other research data (Johnson et al., 1953; Chang and Kummerow, 1953 b).

Phospholipid theory Crude soybean oil contains 1.5-2.5% phospholipids, which are removed to less than 3 ppm during refining. Phospholipids are believed to be antioxidants, chelating agents and synergists with other antioxidants. They, however,

are easily oxidized to give off-colors and off-flavors and develop fishy flavors upon heating (Smouse, 1985).

Caustic refining generally removes virtually all the phospholipids. If soybeans are not harvested promptly after maturity, but are left exposed to rain or damp weather, the seeds may form a dark brown color and chalky texture. This result is termed weather or field damage. If soybeans are stored at moisture levels greater than 13-14%, spontaneous heating usually results in storage damage. The soybean oil from damaged beans may contain a considerable quantity of non-hydratable phosphatides, which are not removed by conventional refining (List, 1980). Evans et al. (1954) added phosphatides to soybean oil prior to deodorization and discovered that their presence caused melon, bitter, and cucumber flavors often found in aged soybean oil. Therefore, any small amounts of phosphatides left in refined soybean oil will contribute to off-flavor development upon oxidation and decomposition (Frankel, 1980 b).

Non-saponifiable/minor component theory Minor nonglyceride components in soybean oil also have been related to reversion flavor. Mattil (1947) added the nonsaponifiable extract of hydrogenated soybean oil to both refined cottonseed oil and refined peanut oil. These oils developed reversion flavors typical of reverted soybean oil.

This theory, however, has been questioned. Hoffmann et al. (1962) added different non-saponifiable extracts of soybean oil to cottonseed oil and found no evidence of reverted soybean oil flavor. Furthermore, reconstituted soybean oils

containing no naturally occurring non-saponifiables also developed typical reversion flavor, which indicated that reversion flavor is not caused entirely by non-saponifiables.

Endo et al. (1988) suggested that reversion flavor in soybean oil is related to trace components in the oil. They studied soybean oil and soybean oil triacylglycerides and found that soybean oil had a much stronger reversion flavor than did soybean oil triacylglycerides. Elsewhere, Endo et al. (1989) found that the precursors or inducers of reversion flavor were present in the minor components eluting with diethyl ether/*n*-hexane (1:1) on a silicic acid column. They, however, still could not identify the flavor compounds. Later, Endo et al. (1991) reported that unusual triacylglycerides in soybean oil were eluted with diethyl ether/n-hexane (1:1) from a silicic acid column, isolated by silica dry gel column chromatography, and purified by thin layer chromatography. Unusual triacylglycerides were characterized by ultraviolet spectrophotometer with hexane as the solvent and by infrared spectrophotometer with CCl_4 as the solvent. Oxo and hydroxy fatty acids isolated from unusual triacylglycerides were identified by gas chromatography and gas chromatography-mass spectrometry as methyl esters. Finally, they believed that 10-oxo-8-octadecenoic acid and 10- and 9-hydroxy octadecanoic acid were responsible for reversion flavor development.

Multivalent metal theory As mentioned earlier, multivalent metals, such as iron and copper, are prooxidants in oils. When they are in a higher state, the multivalent metals can catalyze the decomposition of hydroperoxides, whereas, in the lower state, they can catalyze an oxidation of the unsaturated fatty acid to a

hydroperoxide. Therefore, it is appropriate to conclude that multivalent metals may facilitate the development of reversion flavor (Smouse, 1985).

Light exposure theory Light is very detrimental to the quality of soybean oil. Frankel (1980 b) described two mechanisms by which light can break down soybean oil, including direct photochemical oxidation and photosensitized oxidation. Direct photochemical oxidation is a free-radical reaction catalyzed by ultraviolet irradiation which decomposes hydroperoxides, carbonyl compounds, or oxygen complexes of unsaturated lipids. This reaction, proceeded by a normal chain reaction, can be inhibited or retarded by chain-breaking antioxidants, such as butylated hydroxy anisole (BHA) or butylated hydroxytoluene (BHT) (Frankel, 1980 b).

Photosensitized oxidation is a reaction in which oxygen is activated by visible light in the presence of photosensitizers, such as chlorophylls. This oxidation reacts about 1500 times faster than normal free-radical oxidation and only traces of photosensitizers are sufficient to initiate autoxidation of unsaturated fatty acids. There is enough evidence that sufficient amounts of photosensitizers are left in commercially refined, bleached, and deodorized soybean oil to contribute to its light instability (Frankel, 1980 b).

Other theories There are still some new theories being developed. Recently, Guth and Grosch (1989; 1990 a, b) reported 3-methyl-2,4-nonandione to be a precursor of reversion flavor in soybean oil. This compound has a very low flavor threshold (0.01 ng/L air) compared with other volatile compounds. In 1991, Guth and Grosch proposed two furanoid fatty acids to be precursors of 3-methyl-2,4-nonandione, namely 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid. They detected relatively great amounts (0.02-0.04%) of the two furanoids in both processed and unprocessed soybean oils, and lesser amounts in corn and rapeseed oils (0.0015-0.0035%). The furanoids were not found in olive and sunflower oils.

Controlling flavor reversion

There is no method that can entirely prevent the reversion flavor. Many measures, however, can be employed to retard the development of the reversion of soybean oil. These precautions include adding metal deactivators, ensuring better processing, partly hydrogenating to iodine values of around 110, packaging oils in proper containers, and storing properly in the dark, at low temperatures and under a nitrogen cover (Smouse, 1985; Frankel, 1980 b).

Mechanism of oxidation by lipoxygenase

Characteristics of lipoxygenase enzymes

Lipoxygenase enzymes (E.C. 1.13.11.12) catalyze the reaction of lipids containing a *cis, cis*-1,4-pentadiene system with oxygen to form conjugated hydroperoxides (Figure 3; Chism, 1985), which leads to the development of the off-flavor of soybean oil. The common plant fatty acids which have this structure are 18:2, 18:3, and arachidonic (20:4) acids. Each molecule of lipoxygenase contains one atom of iron, either in the Fe(II) or Fe(III) state during catalysis (Vick and Zimmerman, 1987).

The existence of lipoxygenase was recognized and the enzymes were utilized as early as the 1920's (Sumner and Somers, 1953). Although people did not know the mechanism involved, they knew how to use soy flour to bleach the carotenoid pigments in wheat flour for bread-making. In the United States, enzyme-active soy flour (1% flour weight basis) is usually added to baking flour. The improvement results from the oxidation of the flour lipid by lipoxygenase in the soy flour supplement. The lipid, even though a minor component in the flour, has a definite effect on the mixing and baking properties of the dough (Eskin et al., 1977).

Lipoxygenase enzymes, naturally present in many plants, occur in multiple forms in soybeans. Historically, lipoxygenase has been called the carotene-destroying enzyme (Sumner and Somers, 1953), carotene oxidase, fat oxidase, lipoxidase, and,

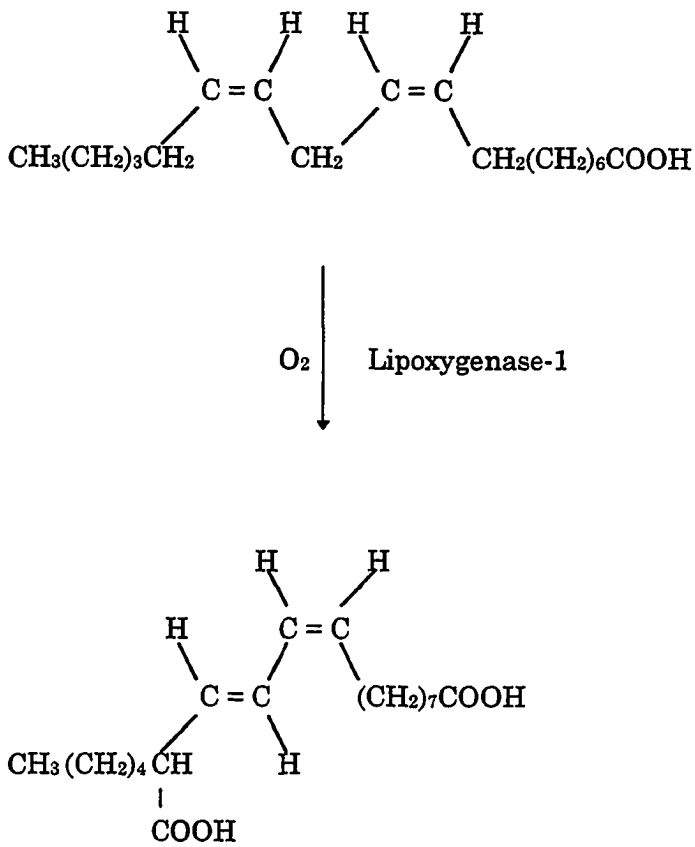

Figure 3. Lipoxygenase-1 catalyzes *cis*-9, *cis*-12-octadecdienoic acid with molecular oxygen to 13-L_S-Hydroperoxy-*cis*-O, *trans*-11-octadecadienoic acid (Chism, 1985)

recently, lipoxygenase (Vick and Zimmerman, 1987). It is recognized that there are two types of lipoxygenases classed mainly on the differences in their pH activity. Type 1 lipoxygenase contains only one isozyme, LOX 1. Type 2 contains two isozymes, LOX 2 and -3 (Vick and Zimmerman, 1987). Although there was a report of the existence of the fourth isozyme (Dreesen et al., 1982), having a pH optimum of 7.5 and an isoelectric point of 5.90, this result has not been confirmed by other researchers. A group of Japanese researchers recently mentioned the presence of at least four kinds of lipoxygenase isozymes in soybean seeds (Endo et al., 1990), but the information source was not given. The main differences (Table 7) of these isozymes are the physical characteristics, such as pH optimum, heat stability, and substrate specificity (Chism, 1985). It is believed that LOX 1 activity is higher with 20:4 and 18:3 than with 18:2 (Zhuang et al., 1991).

Reactions catalyzed by lipoxygenase

As mentioned previously, lipoxygenase isozymes exist in many plants.

Table 7. Characteristics of lipoxygenase isozymes

Characteristics	LOX 1	LOX 2	LOX 3
pI	5.68	6.25	6.15
pH optimum	9.0	6.5	7.0
Substrate specificity (relative rate compared to linoleic acid)			
Methyl linoleate	0.10	0.52	2.0
Linoleyl methane sulfonate	0.17	1.80	1.75
Linoleyl acetate	0.06	0.39	0.75

Source: Chism, 1985

Unfortunately, soybean is the richest source of the enzymes (Faubion and Hoseney, 1981), which greatly affects consumers' acceptability of soy foods (Sessa, 1979). These enzymes have a high degree of specificity for their substrates, the best being a *cis, cis*-1,4-pentadiene group with the methylene group at position eight from the terminal methyl group, but this substrate is not an absolute requirement. The stereospecific removal from the ω-8 methylene group of the hydrogen having the L configuration is the initial step of the reaction, followed by the formation of a hydroperoxy group and rearrangement of the molecule to form conjugated double bonds (Bishop, 1971). The structure of 18:2, which is abundant in the soybean oil, has these structural requirements. Actually, the main substrates for lipoxygenase in higher plants are 18:2 (ω6) and 18:3 (ω3) (Hildebrand, 1989).

Lipoxygenase enzyme catalyzes the reaction of polyunsaturated fatty acids to form hydroperoxides, which, in turn, leads to the formation of off-flavors, such as the grassy-beany and bitter flavors typical of soy products, and the destruction of certain vitamins and proteins (Kitamura, et. al. 1983). Generally, hydroperoxides are formed easily from fatty acid hydroperoxy radicals. The free-radical reaction mechanism is the same as discussed previously. One of the problems is the hexanal production catalyzed by lipoxygenase, which has a very undesirable odor even at very low concentrations (e.g. 5 ppb) (Hildebrand, 1989).

Reaction of the hydroperoxides, however, also may be catalyzed by some enzymes to develop other volatile compounds. The break down of hydroperoxy fatty acids to form the characteristic flavor of certain fruit from which hydroperoxide lyase

comes has been shown to be catalyzed by hydroperoxide lyase (Tressl and Drawert, 1973; Vick and Zimmerman, 1976; and Galliard and Phillips, 1976).

In addition, the break down of hydroperoxides can be catalyzed by hydroperoxide lyase to form aldehydes and oxoacids, by hydroperoxide isomerase to form α- and γ-ketones, and by hydroperoxide cyclase to form cyclized compounds, such as 12-oxo-phytodienoic acid (Vick and Zimmerman, 1987). Actually, in different enzymatic systems, the final products can be different. For example, 12-oxo-phytodienoic acid can be converted to jasmonic acid by the action of 12-oxo-phytodienoic acid reductase and reaction of fatty acid hydroperoxides can be catalyzed to form divinyl ethers by an enzyme system in potato tubers. These reactions are responsible for the development of characteristic flavors of fruits. For instance, reactions of linolenic acid hydroperoxides were catalyzed by extracts of ripening bananas to form volatile aldehydes and oxoacids that contributed to the aroma of bananas (Tressl and Drawert, 1973). The influences of these enzymes and pathways on the soy products, however, are not very clear and still need to be studied.

The physiological role of lipoxygenase in plants has been studied. In general, lipoxygenase is involved in the formation of ethylene from linolenic acid as a precursor and for the biogenesis of flavors of fruits (Eskin et al., 1977; Vick and Zimmerman, 1987) and in both direct and indirect forms of pest resistance in plants (Hildebrand, 1989).

Lipoxygenase and off-flavor

Food quality can be affected by the activity of lipoxygenase. These oxidative products are responsible, at least in part, for the low acceptability of soy products (Davies et al., 1987). In addition, it is possible that the destruction of protein by hydroperoxides might be toxic (Kitamura, et al. 1983). All three isozymes do not contribute equally to off-flavor development. Zhuang et al. (1991) reported that LOX 2 gave the highest C_6 aldehyde production among three isozymes. They also believed that C_6 aldehydes were important in the flavor of many food products and might be responsible in part for the pest resistance of some plants.

To eliminate the influence of lipoxygenase, heat treatment has proven to be effective in improving the quality of soy products (Davies and Nielsen, 1986; Kitamura et al., 1983; Davies et al., 1987; and Frankel et al., 1988), although the solubility of soy protein is decreased by heat, resulting in an unpleasant "cooked" flavor (Kitamura, 1995). Baker and Mustakas (1973) believed the storage stability and palatability of soy flour were increased after the inactivation of lipoxygenase. Furthermore, heat treatment before oil extraction resulted in higher levels of residual oil in the soy meal and higher level of phospholipids in the crude oil (Frankel et al., 1988).

Another alternative to decreasing the effects of lipoxygenase on soy product quality is to develop soybean lines without certain isozymes of lipoxygenases. Hammond et al. (1972) reported research goals at Iowa State University in trying to breed soybean lines with reduced lipoxygenase activity. At that time, it was not known that there are three lipoxygenase isozymes. In 1972, Verhue and Francke reported two

fractions of lipoxygenase isozymes on ion-exchange resins, indicating the presence of two lipoxygenase isozymes. Hildebrand and Hymowitz found two soybean genotypes lacking LOX 1 in 1981 and Kitamura et al. (1983) reported two cultivars lacking LOX 3 in 1983. Recently, Hajika et al. (1991) reported the development of a line lacking all three lipoxygenase isozymes, which was studied for its use as soy milk, to have smaller peaks of volatile compounds according to the gas chromatographic analysis (Kitamura, 1995). The stability of soybean oils from beans lacking some isozymes has been studied (Endo et al., 1990; Frankel et al., 1988; and Kitamura, 1995). Frankel et al. (1988) studied soybean oils from Century 84 and LOX 1 null lines at different refining stages, such as crude, degummed, refined, bleached, and deodorized and concluded that the LOX 1 isozyme did not affect the oxidative stability of soybean oil. Endo et al. (1990) examined soybean oils from lines lacking two lipoxygenase isozymes (LOX 1/LOX 3 null and LOX 2/LOX 3 null) and showed that lipoxygenase in soybean did not affect the oxidative and flavor stability of the refined soybean oil when measuring peroxide value and sensory qualities. But they did notice that the activity of LOX 2 isozyme was correlated with high hexanal production from the soybean seeds. During the preparation of the edible refined soybean oil, soybean seeds were heated to 70 °C, cracked, and then extracted. The heating of the whole soybean seeds obviously denatured the lipoxygenases. Kitamura (1995) also believed the LOX 2 isozyme played a major role in the formation of n-hexanal, the main contributor to the beany flavor. Kitamura (1995) studied volatile compounds by gas chromatography from soy milks of Yumeyutaka, a soybean lacking both LOX 2 and LOX 3, and Kyushu 111, a soybean

lacking all the isozymes, and found that volatile peaks from these two soybean were much lower than that from normal soybeans.

Currently, there are not enough data to fully understand the influence of lipoxygenase isozymes on the stability of soybean oil. A statement made by Wolf in 1975 may still be relevant. Wolf said "lipoxygenase cannot be ruled out as a causative factor until further work clearly demonstrates that lipoxygenase catalysis is not occurring at low moisture levels".

Methods of measuring oxidation

The chemical changes during oxidation of oils are very complicated. Thus, it has been a challenge to determine which methods should be used to measure the extent of oxidation. In the following discussion, many commonly used methods are listed, and advantages and disadvantages are mentioned. The measuring methods are divided into four categories, including physical methods, chemical methods, dynamic methods, and sensory evaluation methods.

Physical methods

Ultraviolet absorption (conjugated diene) Autoxidation of polyunsaturated fatty acids causes a shift in the double bonds, producing a conjugated diene or triene that can be measured by ultraviolet absorption at 232 nm or 268 nm, respectively (Gray, 1985; White, 1991). The official method for this measurement is available as AOCS method Ti-la-64 (AOCS, 1989). Absorption at these wavelengths increases proportionally to oxidation in the early stages but plateaus during frying temperature because of the establishment of an equilibrium between the rate of formation of conjugated dienes and the rate of formation of polymers (White, 1991).

Parr and Swoboda (1976) proposed a different approach for determining the extent of oxidation in foods and fats by using UV spectrophotometry. Both hydroperoxides of polyenoic fatty acids and decomposition products, such as hydroxy and carbonyl compounds, were measured at different wavelengths and expressed as the conjugable oxidation products (C.O.P) value. The peroxide values were measured according to British Standard Method (1958). Another feature of the work of Parr and Swoboda (1976) was the application of the COP ratio, which can be used to distinguish between the oxidation products derived from dienoic fatty acids and those of more highly unsaturated polyenoic fatty acids. An oxodiene value measures the unsaturated carbonyl compounds.

For these measurements, a known weight of lipid (about 0.25 g) is dissolved in a 5.0-mL volume of iso-octane:ethanol (1:1, v:v). One milliliter aliquots of this stock solution are transferred to each of three 25.0-mL stoppered volumetric flasks which are

coded 'O', 'R', or 'D'. The flask coded as 'O' (original) contained 1.0 mL iso-propanol. The flask coded as 'R' (reduced) contained 1.0 mL of a filtered, saturated solution of sodium borohydride in iso-propanol. The flasks are held at 60 °C for 30 minutes and cooled down. The flask coded as 'D' (dehydrated) is first treated as for flask 'R', but 5.0 mL of a 20% (w/v) sulphuric acid solution in ethanol is added and the flask is held at 60 °C for 30 minutes and then cooled down. All three flasks are filled with ethanol to the mark. Three wavelengths (268, 275, and 301 nm) are chosen for measuring absorbancies. The COP ratio is calculated as follows.

Oxodiene value = $(AO_{275} - AR_{275}) \div 0.8w$

COP value = $\{(AD_{268} - AR_{268}) + (AD_{301} - AR_{301})\} \div 0.8w$

COP ratio = $(AD_{301} - AR_{301}) \div (AD_{268} - AR_{268})$

AD is the absorbance of solution D, AR the absorbance of solution R, and AO the absorbance of solution O. W is the weight of lipid in grams. The numbers are wavelengths. Both the oxodiene and COP values can be expressed as absorbance, measured in a 1-cm cell for a 1% w/v lipid solution.

Fluorescence Peroxidizing lipids and cellular constituents, such as certain phospholipids, ribonuclease, deoxyribosenucleic acid and proteins which have free amino groups, can react with each other to form fluorescent compounds, with the general structure of an N,N'-disubstituted 1-amino-3-iminopropene (Gray, 1978). This method was applied mainly for detecting lipid oxidation damage in biological tissues. This procedure was reported to be more sensitive than the TBA test (Gray, 1985).

Volatile compounds With the development of flavor compound isolation techniques and gas chromatography (GC), many papers have been published to evaluate the flavor qualities of oils by GC. GC analyses have been reported to be more reproducible, less time-consuming, less expensive, more simple and less subjective than sensory tests (Min and Kim, 1985). Min (1981) reported that the flavor qualities of vegetable oils during oxidation as monitored by GC correlated very well with sensory analysis, having correlation coefficients of greater than 0.95. GC has proven to be very useful in determining the extent of oxidation in pure mixtures, such as vegetable oils, but it is difficult to identify volatiles in more complex lipid systems, such as foods (Gray, 1978).

Usually, the volatile compounds are isolated and concentrated prior to injection into a GC (Min and Kim, 1985). There are two common ways to isolate and concentrate the volatile compounds. One is to collect and concentrate volatile compounds on an absorbent, such as charcoal, glass-wool or Tenax TA®, and another is to collect and concentrate volatile compounds by cryogenics. Dupuy et al. (1973) proposed a simple, rapid and direct gas chromatographic technique for the examination of volatiles in salad oils and shortenings at the 10 ppb level without prior concentration. In this method, the liner of the inlet of the gas chromatography is packed with volatile-free glass wool to allow slow diffusion of the sample on the glass wool, but to prevent seepage onto the gas chromatographic column. Later, Jackson and Giacherio (1977) modified this method to make it not require special valving and be not limited to a specific sample size. According to the report, the correlation of volatiles with flavor

panel scores was greater than -0.96 with soybean oils aged at room temperature under normal fluorescent lighting. Lee et al. (1995) proposed a dynamic headspace procedure to isolate the volatiles from oxidized soybean oil. The volatiles are trapped in a Tenax TA® tube and desorbed from the absorbent and separated by GC. The main feature of this research is the quantitation of flavor volatiles according to the formula proposed by authors.

Nawar and Fagerson (1960) proposed a method, in which volatile compounds were frozen for collection and concentration. Hornstein and Crowe (1962) modified Nawar and Fagerson's method to get "far more detailed" data. The idea was to utilize a refrigerated, stainless steel or copper coil filled with column material for the collection trap. The Swagelok fittings were used to make this coil an integral part of the column. The chromatogram could be obtained without any gas transfer systems and without the use of heated injection ports, and the collection coil could be used repeatedly without further cleanup.

Hartman et al. (1971) developed a simple procedure, which was widely accepted, for isolation, concentration and analysis of the volatile compounds in vegetable oils. A 400-fold concentration of the volatile compounds was obtained with this method, with a good reproducibility (94.3% to 105.5%). The volatile compounds were isolated by bubbling purified helium through a measured quantity of vegetable oil heated in an oil bath at 177 °C (350 °F). The compounds were collected on activated charcoal and then extracted from the charcoal with carbon disulfide containing an internal standard (Hartman et al., 1971).

Other methods Other physical methods that have been proposed for measuring rancidity in fats and oils but have not been widely used include infrared spectroscopy, polarography and refractrometry (Gray, 1985).

Chemical methods

Acid degree value The acid degree value is obtained by titration. Fat (1 mL) at 57 °C is mixed with 5 mL of fat solvent (4:1 of petroleum ether:n-propanol, v/v) and an indicator. The solution is titrated with standardized alcoholic KOH solution to the end point. The ADV is expressed as mEq/L (Weihrauch, 1988) and should be expressed to the second decimal place only in reporting results (Bianco et al., 1978). Since this method is the most commonly used, it has been widely investigated. Pillay et al. (1980) compared procedures reported by the Bureau of Dairy Industries and by Frankel and Tarassuk and found that ADVs were method dependent. Even different researchers interpret the meaning of ADV differently.

Peroxide value The primary products of lipid oxidation are hydroperoxides, also simply called peroxides. The concentration of peroxides in fats and oils may be used as an indication of the extent of oxidation (Gray, 1985). Hydroperoxides do not have any flavor. When they break down, however, they form carbonyl compounds which have a strong, disagreeable flavor (Weiss, 1983). Thus, peroxide values actually measure the precursors of flavor compounds and only are applicable to the early stages

of oxidation. In the AOCS official method Cd 8-53 (AOCS, 1989), the extent of oxidation is determined by the amount of free iodine the oxidized fat can liberate from potassium iodide. The results are expressed as peroxide value, the milliequivalents of iodine per kilogram of fat. This method is applicable to all common fats and oils.

Another method for measuring the peroxide value is the Stamm method, modified by Hamm et al. (1965). This procedure often is selected because of the very small amount of sample needed. The modified Stamm method is a colorimetric method. The sample (less than 0.5 grams) is mixed with 1,1,2,2-tetrachloroethane and a solution of 1,5-diphenylcarbohydrazide and acetic acid. The whole solution is bubbled, heated and cooled before measuring. The absorbance is then read at 565 nm with a spectrophotometer fitted with constant temperature plates set at 25 °C.

Thiobarbituric acid test (TBA test) The TBA test is a common method for determining the stability of fats and fatty foods, such as meat products. The TBA number (mg malonaldehyde per kg sample) is usually used to express the extent of oxidation by comparing the optical density of the TBA-malonaldehyde colored complex with that of standards from 1,1,3,3-tetraethoxypropane at 532 nm (Gray, 1985). The TBA test is especially useful to compare samples of a single material at different states of oxidation.

TBA-reactive material is believed to be produced only from fatty acids containing three or more double bonds. Various compounds, other than those found in fatty foods, also have been found to interfere with the TBA test by forming colors. Sucrose and some compounds in wood smoke, for example, have been reported to give a

red color when reacted with TBA. Furthermore, malonaldehyde is reported to react with protein to give abnormally low TBA values (Nawar, 1985 a). When this method is used to test unknown samples, particularly foods, care should be taken because of interference in color formation and reaction with components other than malonaldehyde (Jackson, 1981).

Total and volatile carbonyl compounds The total and volatile carbonyl compounds are measured based on the hydrazones derived from the reaction of aldehydes and ketones (oxidative products) with 2,4-dinitrophenylhydrazine (Nawar, 1985 a). The absorption is measured in alkaline medium at 440 nm to give the total carbonyl content, expressed as mmol hexanal/kg fat. In refined, bleached, and deodorized oils, carbonyl values are in the range of 0.5 to 2 mmol/kg (Gray, 1985).

White and Hammond (1983) proposed a carefully designed method to determine the carbonyl compounds in oxidized fats and oils based on formation of trichlorophenylhydrazones. This method was planned to eliminate the interference of hydrocarbons by passing samples through a Florisil column and to minimize the artifacts by using a column of Celite impregnated with stannous chloride. Samples were passed through a column and the eluate was reacted with trichlorophenylhydrazine with Florisil as a catalyst. Trichlorophenylhydrazones were isolated from the samples by chromatography on a Florisil column and quantified by gas chromatography on a 10-m SE 30 capillary column. The method was applied not only to soybean oils stored under various conditions, but also to linoleic acid

hydroperoxide to determine the extent to which decomposition of peroxides gave rise to carbonyl artifacts during the procedure.

The carbonyl compounds, however, also can be produced by decomposition of unstable intermediate substances, such as hydroperoxides, to interfere with quantitative results. Such interference can be minimized by reducing the hydroperoxides to noncarbonyl compounds before determining the carbonyls (Nawar, 1985 a). The major portion of the carbonyl compounds in oxidized lipid is of high molecular weight so a measure of total carbonyl compounds may not directly relate to the rancid flavor (Nawar, 1985 a), even though these high molecular weight compounds may be precursors to volatile compounds (Gray, 1985).

Dynamic methods

Information on the stability of fats and oils under normal processing and handling conditions is necessary for maintaining product quality (Gray, 1985). The measurement of oil stability is intended to depict the length of time a fat or food will remain palatable under conditions encountered during marketing and manufacturing. Several methods may be used to monitor oil stability.

Schaal oven test This method is an accelerated storage test in which the sample, either fat or a fatty food, is put in an oven, usually at a temperature of 60 °C (140 °F), until a certain oxidation level is reached according to a preset amount. Determination of the extent of oxidation is the major difficulty. Usually, a sensory

evaluation panel determines the extent of oxidation (Weiss, 1983) or peroxide values are measured.

Active oxygen method (AOM) This procedure is also called the Swift Stability Test in which peroxide value is used to determine the end point of oxidation. This method has been standardized to an AOCS official method Cd 12-57 (AOCS, 1989). To perform the AOM, the sample is heated in a test tube in a water bath at 97.8 °C (208 °F), while air is blown through the sample. Peroxide values are measured periodically until the sample is sufficiently oxidized or PV reaches the preset number (Weiss, 1983).

Oxygen bomb test The sample (15-30 g) is placed in a sealed container (bomb) at 344.5 - 689 kPa (50 - 100 psi) oxygen pressure, which is put in boiling water for a certain time to reduce the pressure to 13.8 kPa (2 psi) (Cooper et al., 1979; ASTM, 1993). The test is faster than the original AOM and is reported to be more precise and accurate (Nawar, 1985 a).

Sensory evaluation

In the study of oxidative stability of lipids, the data are usually correlated to sensory evaluation, which is the ultimate judgment in flavor evaluation (Jackson, 1981). The application of scientific sensory evaluation for vegetable oils started in the 1940's when the flavor problem of soybean oil was first studied in the United States. Approximately 40 years later, the American Oil Chemists' Society adopted two variations of quality and intensity rating scales as official methods (Warner, 1985). This progress should be merited to the Flavor Nomenclature and Standards Committee, which was founded in 1967 as a sub-committee of the AOCS Standards Committee (Waltking, 1982). Besides the official methods, there are many other published methods for conducting a sensory evaluation. Some of the methods are appropriate for differential tests and some for descriptive analysis. To conduct an effective sensory evaluation, some preparations are needed, which include experimental design, panelists' orientation, sample preparation, and data analysis. Jackson (1981) suggested giving panel members rewards at the end of the tasting period, e.g., cookies or cake, and, if possible, sharing the data to help sustain the interest of the panel members. In addition, realizing the functions and limitations of each method is also beneficial. The most commonly used methods of sensory evaluation are mentioned here.

Triangle test This test is the most commonly used differential method, in which panelists are asked to decide which two of the three samples presented are

identical and which is different. This method can be used for both experienced and inexperienced panelists. Researchers also can use the triangle test for panel training (Warner, 1985). Generally, the triangle test is effective in determining whether an overall difference exists between two samples and has limited use with products that are associated with sensory fatigue, carryover, or adaptation. In a triangle test, the number of panelists is generally greater than twelve, with usual numbers between twenty and forty (Meilgaard et al., 1991).

Line scale test As a descriptive test, the line scale test is commonly used in the area of food science to obtain specified description of the samples (Figure 4). Often, the scale is a line 15 cm long, with or without marks ("anchors") at both ends or at 1.5 cm from the two ends. Typically the left end of the scale refers to none or a zero amount of the stimulus, whereas the right end refers to a large amount or a very strong level of the stimulus. Panelists use the line scale by placing a perpendicular mark on the scale according to their perception of intensity of the attribute being analyzed. The marks from line scales are converted to numbers by measuring the distance of each mark from the left end of the scale either manually or automatically. The advantage of a line scale test is that the intensity can be obtained very accurately because there are no steps or "favorite numbers", and at the same time, the main disadvantage is that it is difficult for a panelist to be consistent because an exact position on a line is not easy to remember (Meilgaard et al., 1991).

AOCS official sensory method An official method for sensory evaluation of vegetable oils is described in AOCS official method Cg 2-83, which provides a standardized technique for the sensory evaluation of edible vegetable oils. In this method, there are two question sheets, which can be used to evaluate flavor quality and flavor intensity of vegetable oils (Figure 5 and 6). Warner (1985) reported that the intensity scale had been successfully used by the northern Regional Research Center to test soybean oils and to correlate flavor evaluations with gas chromatographic volatile analyses. The quality scale was used by the AOCS Flavor Nomenclature and Standards Committee in 1979 for a collaborative test among eight research laboratories to correlate flavor and gas chromatographic volatiles contents in vegetable oils. High correlation coefficients were obtained between flavor and GC data. Attention should be given to the training of panelists. If panelists are not very well trained, the data usually are not acceptable (Warner, 1985).

NAME _____

DATE _____

INSTRUCTIONS

You will be given a blank emulsion, a standard emulsion, and several sample emulsions. The sample emulsions will be in random order. There may be duplicates included in the samples. You are to compare the off-flavor intensity of the samples.

Place a slash perpendicular to the line scale at the point which best describes your evaluation of the attribute. Write the code number of each sample on its slash.

OFF-FLAVOR

---

weak middle strong

COMMENTS:

Figure 4. An example of a line scale evaluation sheet

Name _____ **Date** _____

Directions: Take 5 to 10 mL of warm oil into the mouth, pull air through the oil and evaluate the flavor by exhaling through the nose. Mark off on the intensity scale below the overall flavor intensity for the sample.

Quality	Overall Quality Scores Sample Numbers			Descriptions	Description Intensity Sample Numbers		
10 Excellent				Nutty			
9 Good				Buttery			
8				Corny			
7 Fair				Beany			
6				Hydrogenated			
5 Poor							
4				Burned			
3 Very Poor				Weedy			
2				Grassy			
1				Rubbery			
				Melon			
				Painty			
				Fishy			
				Other			

Figure 5. Scoresheet for flavor quality evaluation

Name _____ **Date** _____

Flavor Intensity Evaluation

Directions: Take a small bite of bread cube into the mouth and chew for a few times, pull air through the sample and evaluate the flavor by exhaling through the nose. Mark off on the intensity scale below (left) the overall flavor intensity for the sample. Then from the master list of descriptions (right) list the one(s) appropriate for this sample and indicate the amount of flavor as weak, moderate, or strong.

Intensity	Overall Intensity Scores Sample Numbers			Descriptions	Description Intensity Sample Numbers		
	_____	_____	_____		_____	_____	_____
10 Bland	_____	_____	_____	Nutty	_____	_____	_____
9 Trace	_____	_____	_____	Buttery	_____	_____	_____
8 Faint	_____	_____	_____	Corny	_____	_____	_____
7 Slight	_____	_____	_____	Beany	_____	_____	_____
6 Mild	_____	_____	_____	Hydrogenated	_____	_____	_____
5 Moderate	_____	_____	_____	Burned	_____	_____	_____
4 Definite	_____	_____	_____	Weedy	_____	_____	_____
3 Strong	_____	_____	_____	Grassy	_____	_____	_____
2 Very Strong	_____	_____	_____	Rubbery	_____	_____	_____
				Melon	_____	_____	_____
1 Extreme	_____	_____	_____	Painty	_____	_____	_____
				Fishy	_____	_____	_____
				Other	_____	_____	_____

Figure 6. Scoresheet for flavor intensity evaluation

OXIDATIVE STABILITIES OF SOYBEAN OILS WITH ELEVATED PALMITATE AND REDUCED LINOLENATE CONTENTS

A paper to be submitted to the Journal of the American Oil Chemists' Society

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ABSTRACT

Soybean lines developed to contain different amounts of palmitate (16:0) and linolenate (18:3) were evaluated for oxidative stability. Soybean oils were extracted in the laboratory from the soybean seeds and refined, bleached, and deodorized. Two replications separated at the point of conditioning were evaluated for each genotype, including Hardin 91 (normal beans), P9322 (10.6 % 16:0 and <2.6 % 18:3), A91-282036 (26.3 % 16:0 and 9.8 % 18:3), and HPLL (23.2 % 16:0 and 3.5 % 18:3). The fatty acid compositions, peroxide values, and cloud points were measured. Elevating 16:0 and/or lowering 18:3 increased the oxidative stability of soybean oils. Soybean oils with elevated 16:0 had higher solidification temperatures than did oils with normal 16:0 content, and soybean oils with low 18:3 content had higher solidification temperatures than did oils with normal 18:3 contents.

KEY WORDS: hydrogenation, peroxide values, oxidative stability, soybean oils, and *trans* fatty acids.

INTRODUCTION

Soybean oil is not stable to oxidation, especially at high temperatures, because of its relatively high content of polyunsaturated fatty acids. In the soybean oil industry, the most commonly used way to increase the oxidative stability of soybean oil is via partial hydrogenation. Hydrogenation introduces *trans* fatty acids (*t*FAs): which are fatty acids in which the alkyl groups surrounding a double bond are on geometrically opposite sides of the molecule (Nawar, 1985). The *t*FAs have physical properties similar to those of saturated fatty acids (Gurr, 1983; Dupont et al., 1991), and higher melting points than other fatty acids with the same carbon number, and with *cis* double bonds.

Mensink and Katan (1990) reported that consuming *t*FAs enhanced low-density lipoprotein (LDL) cholesterol and lowered high-density lipoprotein (HDL) in human plasma, which heightened public concerns about hydrogenation of vegetable oil. Mag (1994) noted that margarine consumption in Canada has declined at least partly because of the *t*FA issue. Although health concerns regarding consumption of *t*FAs have been questioned, there are researchers who support this finding (Zock and Katan, 1992; Lichtenstein, 1993; Wahle and James, 1993).

The level of *t*FAs in the U.S. diet has been estimated to be from 8.1 g/capita•day (Hunter and Applewhite, 1991) to 13.3 g/capita•day (Enig et al., 1990). Enig et al. (1990) reported that 91.3% of *t*FAs was from vegetable oils. Gurr (1983) believed the average intake of *t*FAs was 12 g/capita•day, with 95.2% coming from partially hydrogenated vegetable oils. Because soybean oil accounts for about 75% of all visible oil consumption in the United States, partially hydrogenated soybean oil is the main source of *t*FAs in the U.S. diet.

Because of concerns over *t*FAs, the U.S. Food and Drug Administration (FDA) is considering labeling *t*FA in foods (Haumann, 1994). The FDA is reviewing whether to require labels of processed foods to include information on dietary *t*FA contents (Anderson, 1995). Without hydrogenation, one way to enhance the oxidative stability of soybean oil is to increase its content of saturated fatty acids and to decrease the polyunsaturated fatty acid contents by breeding. Researchers at Iowa State University have developed soybean lines containing elevated palmitate (16:0, 23-25% vs 11.0% as normal) (Fehr et al., 1991) with different linolenic acid (18:3) contents (2.7-9.3%) (Graef et al., 1988). Naturally saturated oils also would have fewer processing costs and should result in more profit for the farmers and/or less cost for the consumers. Miller and White (1988 a, b) reported that oil from the soybean line, A6, containing 20% stearate (18:0), was more stable than oils from commercial cultivars and several other studies have shown superior oxidative stability of soybean oils with low linolenic acid content (Liu and White, 1992). Soybean oils with high palmitic acid and reduced linolenic acid contents may also have greater oxidative stabilities than traditional soybean oils.

Normal soybean oil tends to have *beta* type crystallization, which is not suitable for producing shortenings or margarines (Brekke, 1980). Generally, the *beta*-type oils have relatively low 16:0 (about 10%), whereas the *beta-prime*-type fats, which produce fats with smooth crystal structures, contain at least 20% of 16:0 (Wiedermann, 1978). Because the 16:0 content in soybean oils with elevated 16:0 contents are over 23%, it is possible that the new soybean oils may have *beta-prime* type crystallization for making high quality shortenings or margarines. The objectives of this study were to investigate the oxidative stability of soybean oils with combinations of high 16:0 and either normal (~8%) or reduced (~3%) 18:3 contents.

MATERIALS AND METHODS

The commercial soybean cultivar (Hardin 91), a low-linolenic acid (18:3) and normal palmitic acid (16:0) soybean cultivar (P9322), an elevated 16:0 and normal 18:3 soybean line (A91-282036, abbreviated as A91), and an experimental soybean line containing high 16:0 and low 18:3 (HPLL) were grown near Ames, IA, in 1993 (Table 1).

Despite attempts to match the 16:0 contents of A91-282036 and HPLL and the 18:3 contents of P9322 and HPLL, these values were not the same because of genetic differences among the genotypes. The individual and combined influences of 16:0 and 18:3, however, could still be studied in the oils extracted from these beans.

Oil extraction The moisture content of the soybean was measured by using a Gac2000 Grain Analysis Computer (Dickey-John Corporation, Auburn, IL). To equalize moisture contents among all soybean lines, moisture was adjusted to 11% by spraying the appropriate amount of water over the beans, thoroughly mixing them and storing them over night at 5 °C. Random moisture evaluations of the beans after storage indicated homogeneity in moisture distribution. Samples from each genotype were divided into two lots after moisture conditioning to give two replications for each of the four soybean genotypes.

The extraction method (See Figure 1) was developed from a method described by Hassanen (1985) and Reuber (1992). Each replicate of flakes (0.70 kg) was put into two vessels and extracted simultaneously at 60 °C and at a ratio of 2:1, hexane:flakes, in the laboratory extraction simulator. During extraction, miscella was pumped to vessels containing flakes through which it percolated. The pumping rate was controlled to maintain 1.0 cm of miscella over the flakes. Flakes from each replicate were extracted three consecutive times (stages) with fresh hexane for 6 min of recirculation, plus 3 min for draining. The miscella collections from the three stages were pooled. The time interval from cracking to extracting was kept between 45 to 50 min for each extraction.

The miscella was desolventized in a rotary evaporator (Wheaton, Heidolph, W. Gemany) at 60 °C at 80 ppm. For each replicate, 2 h was needed for desolventization. After desolventizing, the crude oils were stored at -14 °C under nitrogen until needed. The crude oil yields (in percentage of flake weight) were 23.3% for Hardin 91; 23.5% for P9322; 19.2% for A91; and 19.4% for HPLL.

Refining, bleaching, and deodorization Crude oils from each replicate were refined, bleached, and deodorized before being used in storage stability tests as described by Shen et al. (submitted). The yields of refined, bleached, and deodorized (RBD) soybean oils were 78.7% of crude oil for Hardin 91; 75.1% for A91; 74.2% for P9322; and 78.4% for HPLL.

Accelerated stability tests at 60 °C in the dark Accelerated stability tests were conducted at 60 °C. Eight replicate oil samples (0.040 kg) were stored in 100-mL beakers without covers at 60 °C in the dark until sufficiently oxidized. Every another day, an aliquot of soybean oil was removed for analysis. Analyses on oil replicates were measured twice and averaged.

Peroxide values (PV) were measured according to the Stamm method as modified by Hamm et al. (1965). Triacylglycerides were converted into fatty acid methyl esters (FAME) according to a method described by Hammond (1991). The FAMEs were injected onto a Hewlett-Packard 5890 Series II gas chromatography (Kennett Square, PA) equipped with a flame ionization detector and split/splitless injector. A DB-23 fused-silica capillary column was used with dimensions of 0.25 mm × 15 m × 0.25 μm film thickness (J&W Scientific Inc., Rancho Cordiva, CA). Chromatographic parameters were set as follows: injector temperature 250 °C, detector temperature 250 °C, column temperature 200 °C, and carrier gas (helium) flow rate 100 mL/min.

Cloud point test A cloud point test was performed according to modifications of AOCS official method Cc 6-25. An aliquot (15g) of each of the eight soybean oil replicates was placed in a 30-mL beaker. Each sample was heated to 130 °C then cooled to 50 °C before being placed in an ice-water bath. During cooling in the water bath, the oil was stirred with a thermometer to prevent uneven crystallization of the oil. The end point was determined when the thermometer could not be seen at the back of the beaker when viewed horizontally.

Statistical analysis A randomized 2 x 2 factorial design was used for this experiment. Data from all treatments were analyzed by standard analysis of variance procedures (SAS, 1985). Differences in mean values among treatments were determined by the least significant different (LSD) test at $P = 0.05$ (SAS, 1985).

RESULTS AND DISCUSSION

The fatty acid compositions of the soybean oils are listed in Table 1. After storage at 60 °C in the dark, the fatty acid compositions showed a slight decrease in the relative percentages of the polyunsaturated fatty acids and a slight increase in the relative percentages of 16:0 (Table 1). Similar changes in fatty acid contents during storage of soybean oils also were observed by other researchers (White and Miller, 1988; Liu and White, 1992). During storage of soybean oils, polyunsaturated fatty acids were oxidized, resulting in the decrease of their relative percentage contents.

After day 8, soybean oils with reduced 18:3 and elevated 16:0 contents (P9322, A91, and HPLL) had significantly greater PVs than did the oils with normal 18:3 and 16:0 contents (Hardin 91) (Table 2). After day 14, soybean oil with reduced 18:3 content (P9322) had PVs similar to those of soybean oil with high 16:0 content (A91). The commercial soybean oil, Hardin 91, with normal 18:3 and 16:0 contents, tended to have greater PVs than did the oils with low 18:3 (P9322) or high 16:0 (A91-282036) contents after 8 days of storage. Both White and Miller (1988) and Liu and White (1992) reported that soybean oil with elevated stearate content had a significantly greater oxidative stability than did normal soybean oil, and soybean oils with low 18:3 contents oxidized at significantly lower rates than did oils with normal 18:3 contents.

In evaluating canola oils with different fatty acid compositions, Neff et al. (1994a) noted that the oxidative instability of canola oil was positively correlated with the amount of 18:3 content. Neff et al. (1994b) studied the oxidative stabilities of blends and interesterified blends of soybean oil and palm olein and concluded that oxidative stability was improved by lowering the 18:3 content.

The calculated oxidizability (Table 1) of the soybean oils suggested the order of oxidation to be Hardin 91 > A91 > P9322 > HPLL. In this study, the PVs of the oils during storage came very close to these predicted values. A91 tended to have slightly lower PVs than did P9322; however, these differences were significant only on day 10. Because the oxidizability of soybean oil is based on the percentage contents of unsaturated fatty acids, the influence of different 16:0 contents of soybean oils was not directly reflected in the calculated oxidizability. Because of the high 18:3 content (9.8%), A91 had the second greatest calculated oxidizability, however, A91 tended to

have a slightly lower PV than P9322. P9322 had the greatest 18:2 content, whereas A91 had the lowest 18:2 content.

The soybean oils were evaluated by a modified cloud point test to characterize their potential use as refrigerated salad oils (Table 2). For a given fatty acid composition, the cloud point temperature is usually constant and depends mainly on the amount of saturated fat present (Mehlenbacher, 1960). The four soybean oils were liquid at room temperature. The commercial soybean oil, with normal 16:0 and 18:3, had a significantly lower cloud point than did the other oils. The other three soybean oils, with either greater 16:0 contents or lower 18:3 contents, or both, had cloud points that were very close to each other. This information was in agreement with the PV measurements, except for HPLL.

Wiedermann (1978) noted that generally the *beta-prime-type* fats have at least 20 % 16:0. In this study, both A91 and HPLL had over 20 % 16:0. Thus, these two soybean oils should form *beta-prime-type* crystals, especially after interestification, which would make the oils suitable for the production of margarines and shortenings. This hypothesis should be evaluated.

ACKNOWLEDGMENTS

The research was partly funded by the Iowa Soybean Promotion Board and by a grant from the United States Department of Agriculture (USDA) for research programs of the Center for Designing Foods to Improve Nutrition. Soybean oil processing and extraction took place in the Center for Crops Utilization Research (CCUR) at Iowa

State University. This is Journal Paper No. J-xxxxx of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Project No. xxxx).

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Table 1. Calculated oxidizability of soybean oils and fatty acid composition (relative area %) of soybean oils extracted from laboratory-scale extraction before and after storage at 60 °C in the dark for 32 days

		Fatty acid composition by GLC (%)[a]					Calculated
Soybean oil	Status	16:0	18:0	18:1	18:2	18:3	oxidizability[b]
Hardin 91	start	10.3	5.2	25.2	52.0	7.4	7.2
	end	10.8	5.0	25.3	51.8	7.2	
P9322	start	10.6	4.7	26.4	55.5	2.6	6.5
	end	11.0	5.0	26.9	54.7	2.5	
A91-282036	start	26.3	4.5	15.0	44.4	9.8	6.8
	end	27.1	4.4	15.2	43.8	9.4	
HPLL	start	23.2	4.8	21.6	47.3	3.5	5.8
	end	23.4	4.8	21.4	47.1	3.4	

[a] Values are the average of duplicate analysis of two replications.

[b] Oxidizability = [oleate% + 10.3 (linoleate%) + 21.6 (linoleneate%)]/100 (Fatemi and Hammond, 1980)

Table 2. Peroxide values (meq/kg) and cloud points of soybean oils during storage at 60 °C in the dark

Day	Soybean oil genotypes			
	Hardin 91	P9322	A91	HPLL
Peroxide Value				
0	0.2[a]	0.2[a]	0.1[a]	0.2[a]
2	0.3[a]	0.3[a]	0.3[a]	0.3[a]
4	0.5[a]	0.4[a]	0.4[a]	0.4[a]
6	0.7[a]	0.6[a]	0.6[a]	0.7[a]
8	1.7[a]	1.1[a]	0.9[a]	0.8[a]
10	7.8[b]	5.1[b]	1.4[a]	1.2[a]
12	16.2[b]	10.5[b]	4.1[a]	3.1[a]
14	24.9[b]	16.2[ab]	11.5[a]	7.0[a]
16	35.0[b]	24.3[ab]	23.3[ab]	13.9[a]
18	45.5[c]	36.1[bc]	34.8[b]	18.5[a]
20	60.8[b]	45.7[ab]	43.1[ab]	24.7[a]
22	71.7[b]	54.7[ab]	55.4[ab]	35.2[a]
24	79.2[b]	62.9[ab]	69.2[ab]	43.7[a]

[a-b] Values in the same row with different superscripts were significantly different ($P \leq 0.05$).

Table 2. (Cont.)

26	94.1[b]	89.2[b]	83.1[b]	47.5[a]
28	110.0[b]	99.3[ab]	98.5[ab]	59.9[a]
30	139.2[b]	114.2[ab]	111.5[ab]	73.8[a]
Cloud Point (°C)				
	2.5[a]	3.9[b]	4.0[b]	4.1[b]

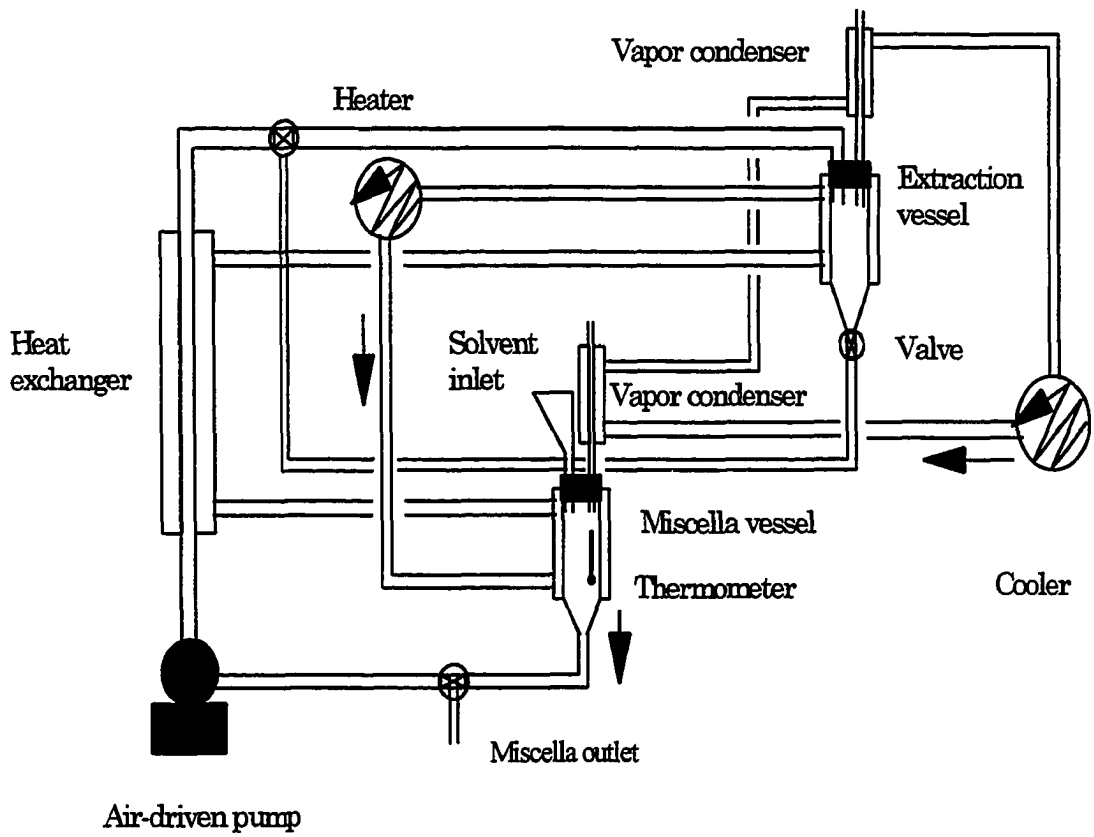

Figure 1. Diagram of laboratory-scale extraction apparatus for extracting oil with hexane from soybean flakes

OXIDATIVE STABILITIES OF SOYBEAN OILS LACKING LIPOXYGENASES

A paper to be submitted to the Journal of the American Oil Chemists' Society

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ABSTRACT

Lipoxygenase (LOX)-null soybean lines lacking LOX 2, LOX 2 and 3 and containing normal (8.0 - 8.6%) or low (2.0 - 2.8%) linolenate (18:3) amounts were evaluated for their oil qualities and storage stabilities. Soybean oils of six genotypes were extracted by both laboratory-scale and pilot-plant scale systems and were refined, bleached, and deodorized in the laboratory. Citric acid was added to oils during the cool-down stage of deodorization. Two replications separated at the point of conditioning were evaluated for each genotype. Under storage conditions of 55 - 60 °C in the dark, soybean oils with low 18:3 contents were significantly more stable as measured by peroxide values than were oils with normal 18:3 contents, regardless of the LOX contents of the beans. The volatile analysis showed few differences between oils with low and high 18:3 contents or among oils from beans lacking different LOX enzymes. After 16 days of storage, the amount of 1-octen-3-ol was significantly greater in oils with low 18:3 content, and soybean oils from beans with normal LOX content had a significantly lower amount of 1-octen-3-ol than did the oils lacking LOX enzymes. Storage at 35 °C under light showed no

differences in peroxide values, volatile amounts, or sensory evaluations after 14 days of storage. Generally, the absence of LOX 2 or LOX 2 and 3, although having a small effect on lipid oxidation, was not as important to oil quality as was the 18:3 content.

KEY WORDS: Autoxidation, fatty acids, hydroperoxide, lipoxygenase, peroxide value, sensory evaluation, soybean oil, and volatile compounds.

INTRODUCTION

The flavor instability of soybean oil has been a challenge to the food industry. The relatively high linolenic acid (18:3) content (8%) is believed to be the major contributor to the development of off-flavor. Recently, mutation breeding has been used to change the fatty acid composition of soybean oil and increase its stability. Previous research showed that soybean oil with low 18:3 (3%) was more stable than soybean oil with normal 18:3 (8%) contents (Miller and White, 1988; Liu and White, 1992). Currently, soybean oil with low 18:3 content is commercially available (Anonymous, 1994).

In addition to the fatty acid composition, lipoxygenase (LOX) enzymes are also thought to contribute to the flavor instability of soybean oils (Kitamura et al., 1983; Davies and Nielsen, 1986; Davies et al., 1987; Frankel et al., 1988; White, 1992). LOX includes a group of enzymes which catalyze the hydroperoxidation of polyunsaturated fatty acids or esters containing a *cis, cis*-1,4 pentadiene system (Eskin et al., 1977; Hildebrand et al., 1991). The abundant amount of polyunsaturated fatty acids in

soybean oil, such as linoleic acid (18:2) and 18:3, provides substrate for this enzyme. LOX has three isozymes, named LOX 1, LOX 2, and LOX 3. The main differences in the activities of these isozymes include pH optimum, heat stability, and substrate specificity (Chism, 1985).

Genetic removal of the LOX 2 isozyme reduced off-flavors in soy foods, such as soy milk (Davies et al., 1987), thus, improving acceptability of soy foods because off-flavor is the major factor restricting the use of soy foods (Rackis et al., 1979). Frankel et al. (1988) found that the absence of LOX 1 did not affect the oxidative stability of soybean oil. Endo et al. (1990) studied the oxidative stability of soybean oil from LOX 1- and 3-null and LOX 2- and 3-null beans. The oils were stored at 30 °C under light (100 lux). Sensory evaluations and peroxide values measured on days 1, 3, 5, and 7 showed no influence of these combinations of missing LOX on oxidative stability. Soybean oils from beans lacking additional combinations of the LOX isozymes may have better oxidative stability than the LOX-null soybean oils already tested and from beans with normal LOX enzyme content.

The objectives of this study were to compare the oxidative stabilities of soybean oils with normal and reduced LOX contents, in combination with normal and reduced 18:3 to determine their influences on oil stability. The influence of LOX 1 was not studied because beans lacking this isozyme were not available.

MATERIALS AND METHODS

Commercial soybeans (Century 84), a low-linolenic (18:3) soybean genotype (A89-269043) and four experimental soybean genotypes were grown near Ames, IA in 1992. After harvest, the soybean seeds were grouped according to 18:3 and lipoxygenase (LOX) contents, as described later. Century 84 and A89-269043 were grown as pure genotypes with known LOX and 18:3 contents.

Experimental soybean genotypes lacking one or two LOXs were developed in the Department of Agronomy, Iowa State University. The plant cross method is described briefly: The first filial generation seeds were obtained from the cross AX7788 (L2L3-2-4 x A89) in Ames in 1990. L2L3-2-4 is a genotype lacking LOX 2 and 3, which was obtained from Purdue University and A89 is a 2% 18:3 genotype from the 1989 2% 18:3 soybean genotype. The plants of the second filial generation were grown and harvested individually. Individual plants were screened for LOX by using a rapid spot test designed by Hammond et al. (1992). Plants of the second filial generation missing LOX 2 and LOX 2 and 3 were analyzed for fatty acid composition according to a method described by Hammond (1991) in which the triacylglycerides are converted into fatty acid methyl esters (FAMEs) and injected onto the gas chromatography (GC). Theoretical response factors for quantitating the fatty acids were applied to the results as proposed by Ackman (1992). Selected plants were pooled and planted. Individual third filial-generation plants were analyzed for fatty acid contents and LOX contents, and again were pooled as LOX 2 or LOX 2 and 3. All tested soybean seeds were pooled into six categories according to 18:3 and LOX contents (Table 1).

Screening for lipoxygenase. The beans were analyzed for LOX content indicating the presence or absence of LOX 2 and LOX 3 or both (Hammond et al., 1992). Soybean seeds were crushed in a hydraulic press to allow the LOX to react with added pure soybean oil. Hydroperoxides formed during this step, because of the presence of LOX reacted with ferrous sulfate and ammonium thiocyanate to form color.

Oil extraction. The moisture content of each replication of soybeans was measured by using a Gac2000 Grain Analysis Computer (Dickey-John Corporation, Auburn, IL). To equalize moisture content for all beans, moisture was adjusted to 11% by spraying the appropriate amount of water over each group of beans, thoroughly mixing them, and storing them at 5 °C. The storage time was overnight (12 h) for laboratory-extracted beans and two days for the greater quantity of beans used in pilot plant extraction. Random moisture determinations of small amounts of beans after storage indicated homogeneity of the moisture distribution.

For each type of extraction, soybean seeds were then cracked, heated, and flaked. Soybean seeds were cracked into six to eight pieces in a corrugated roller mill (Ferrell-Ross Co., Oklahoma City, OK). For laboratory extraction, the cracked seed was heated within 20 min to 70 °C in a 10-gal steam kettle (Lee Metal Products Co. Inc., Philipsburg, PA). For pilot-plant extraction, the greater quantity of cracked soybean seeds was heated to 70 °C in a three-tray seed conditioner (French Oil Mill Machinery Co, Piqua, OH). Heated and cracked soybean seeds then were flaked in a smooth roller mill (Roskamp Manufacturing Inc., Waterloo, IA) to about 0.25 mm in thickness. For

each extraction method, samples from each genotype were divided into two lots prior to moistening to give two replications for each of the six soybean genotypes. Soybean flakes were extracted by two methods, laboratory-scale and pilot-plant-scale extraction, to examine the possible influence of extraction methods on the oil stability.

Laboratory-scale extraction. Soybean flakes from each genotype were extracted in duplicate, to give twelve total sample extractions. The extraction method was described in previous paper (Shen et al., submitted).

The miscella was desolventized in a rotary evaporator (Wheaton, Heidolph, W. Gemany) at 60 °C at 80 rpm. For each 0.70 kg of miscella, 17 min was needed to desolventize. After desolventizing, the crude oils were stored at -14 °C under nitrogen until needed.

Pilot-plant scale extraction. Soybean seeds (11.4 kg) from each genotype were extracted on pilot plant scale in duplicate, to give twelve total samples. Each replicate (9.1 Kg flakes) was extracted at 60 °C, with a ratio of solvent to flakes of 1.3 to 1 (per stage). During each extraction, the flakes were extracted in a batch-advance solvent-extractor (French Oil Mill Machinery Co., Piqua, OH) in five stages with 10 min of extraction, plus 3 min of draining between stages.

The miscella was desolventized and the meal was toasted in a three-tray desolventizer toaster (French Oil Mill Machinery Co., Piqua, OH). The miscella was heated to 220 °F at 330.2 mm of Hg vacuum, which was maintained for 5 min. The resulting crude oils were stored at -14 °C under nitrogen.

Refining, bleaching, and deodorizing. Crude oils from each replicate from both types of extraction were refined, bleached, and deodorized before being used in storage stability tests.

Refining. The free fatty acid (FFA) contents of the crude oils were determined according to AOCS official method Ca-5a-40 (1989). An amount of NaOH was added to each oil according to AOCS official method Ca-9d-52 (1989) and stirred for 90 min at 25 °C, followed by heating to 65 °C for 20 min with stirring and then holding for 1 h at 65 °C. Finally, the oil was allowed to sit for a minimum of 12 h at room temperature. Soapstock was removed by filtration.

Bleaching. The alkali-refined oils were bleached by using the AOCS official method Cc-8b-52 (1989). Official natural bleaching earth (3%) was added to the oil, the mixture was heated to 120 °C for 5 min and filtered immediately by using Fleaker® filtration units (Spectrum, Houston, Texas) with Whatman No. 1 filter paper.

Deodorizing. The bleached oils were steam deodorized by high vacuum (<0.5 Torr) and high temperature (230 to 240 °C for 2 h). The steam distillation method was described by Stone and Hammond (1983) and modified according to Moulton (1989). When the oil was cooled to 100 °C, 100 ppm citric acid was added. Duplicate sets of each oil were refined, bleached, and deodorized, separately. The oil was stored under nitrogen at -14 °C until needed.

Experimental design of oxidative stability tests. Oils were evaluated for oxidative stability in three separate studies.

Study 1: Storage of laboratory-extracted oils at 60 °C in the dark. Twelve replicate oil samples (40 g each) were stored in 100-mL beakers without covers in the dark at 60 °C for 12 days. On every third day, an aliquot of soybean oil was removed after mixing for analysis. Oils were tested in duplicate and each duplicate was measured for peroxide value (PV) two times and the results were averaged.

PVs were measured according to the Stamm method as modified by Hamm et al. (1965). Fatty acid compositions of the oils were measured before and after storage by the procedure previously described. The oxidizability was calculated for each oil according to a formula proposed by Fatemi and Hammond (1980). Oxidizability = [oleate% + 10.3 (linoleate%) + 21.6 (linolenate%)]/100.

Study 2: Storage of pilot-plant-extracted oils at 50 °C in the dark. Twelve replicate oil samples (200 g each) were stored in 250-mL beakers without covers at 50 °C in the dark for 22 days. Every other day, an aliquot of soybean oil was removed for analysis of PVs, FAMEs, and volatile compounds. Volatile compounds were analyzed according to a method proposed by Lee et al. (1995). For each oil sample, volatile compounds were measured in duplicate on day 0 and day 16.

Study 3: Storage of pilot-plant-extracted oils at 35 °C under light. Twelve replicate soybean oils (40 g) were stored in 100-mL beakers without covers at 35 °C under fluorescent light for 14 days. Light intensity, measured by a light-level meter (Weston Illumination Meter, Weston Instruments, Division of Daystrom, Inc., Newark, NJ), was 160 foot-candles. PVs were analyzed every other day. For each replicate,

volatile compounds and FAMEs were measured in duplicate on both days 0 and 14. Sensory evaluations were performed at the beginning and at the end of storage. Panelists were trained during four practice sessions with both fresh and oxidized soybean oils to familiarize panelists with samples similar to those they would be evaluating and with the evaluation methods. Sensory evaluations were performed by using the emulsion method described by Dixon and Hammond (1984). The emulsion was a mixture of 10 g of oil (1%), 6.5 g of gum acacia (0.65%), and 1 kg of water. A line-scale questionnaire was used, in which the scale was a line 15 cm long, without marks ("anchors") at both ends. The left end of the scale referred to the weakest off-flavor, whereas the right end referred to the strongest off-flavor. Panelists used the line scale by placing a perpendicular mark on the scale according to their perceptions of intensities of off-flavors. The marks from the line scales were converted to numbers by measuring the distance of each mark from the left end of the scale. Each replicate oil sample was evaluated two times by the sensory panel on day 0 and again on day 14.

Statistical analysis. A randomized 2 x 3 factorial design was used for this experiment. Data from all treatments in each storage test were analyzed by using ANOVA and LSD for statistical significance (SAS, 1985). The main plot was represented by 6 treatments (2x3), plus 2 replicates.

RESULTS AND DISCUSSION

The fatty acid compositions of soybean oil types studied are shown in Table 2. The Century 84 (#1) and A89-269043 (#4) served as controls but had less 18:3 and more 18:1 than did the other oils in their respective group. The calculated oxidizabilities of these two oils were lower than the others within the group. These fatty acid differences may reduce the impact of eliminating the LOX isozymes. The soybean oils used in all studies were from the same seeds.

Study 1: Storage of laboratory-extracted oils at 60 °C in the dark The PVs of all soybean oil types during storage for 12 days are shown in Table 3. PVs were significantly different between 18:3 groups for all days, except days 3 and 12. There were no significant differences in PVs among the LOX groups. Values for individual soybean oil groups also suggested the same trends. These data indicate that a low 18:3 content is important to oxidative stability of soybean oil, but that removal of LOX 2 or of LOX 2 and 3 had no significant effect. Other researchers have shown similar effects on stability of soybean oils with a reduction of 18:3 (White and Miller, 1988; Liu and White, 1992).

The fatty acid profiles were determined before and after oxidation (Table 2). Generally, the contents of polyunsaturated fatty acids tended to decrease because of oxidation. Oxidizability was calculated from the fatty acid composition, as proposed by Fatemi and Hammond (1980). The PVs of oils with the lowest calculated oxidizability (#4, #5, and #6) were significantly lower than oils with the highest calculated

oxidizability (#1, #2, and #3) (Table 3). Differences in calculated oxidizability among oils with similar 18:3 contents, but from beans with different isozyme contents, were minimal, except for #4 (value of 6.0) which may have had a slight influence on oil stability.

Study 2: Storage of pilot-plant-extracted oils at 50 °C in the dark The stabilities of soybean oils from pilot-plant extractions were evaluated at 50 °C, to be consistent with the temperature used to collect the volatile compounds. The PVs confirmed that soybean oils with low 18:3 (#4, #5, and #6) were more stable than were those with normal 18:3 content (#1, #2, and #3) (Table 4). These differences were significant after 6 days of storage. Because larger samples (200 g instead of 40 g) and lower storage temperature (50 °C instead of 60 °C) were employed in Study 2 than in Study 1, the development of oxidation was much slower in Study 2 than in Study 1. From day 2 to day 12 of storage, there was a tendency for soybean oils from beans lacking LOX 2 isozyme (#2 and #5) to have the highest PVs, whereas the oils from beans without LOX 2 and 3 (#3 and #6) had the lowest PVs. This tendency was significant on days 2, 8, and 10. From day 16 to 22, soybean oils from beans having normal LOX content (#1 and #4) tended to have the lowest PVs. On days 20 and 22, this difference was significant. After 14 days of storage, soybean oils lacking LOX 2 and 3 (#3 and #6) tended to have the highest PVs. These differences in LOX group were not observed in Study 1. The calculated oxidizabilities suggested that #3 and #6 (and even #2 and #4) might be less oxidatively stable than #1 and #5 (Table 2).

The fatty acid compositions before and after storage were measured (Table 2). Because the oils were not oxidized severely, the ending values were not substantially different from the beginning values. This observation also was noted by White and Miller (1988). When PVs were less than 30 meq/kg, the ending values of 18:3 were close to the beginning values.

Volatile compounds were analyzed by GC on days 0 and 16 (Table 5). A total of nine volatile compounds were chosen for measurement based on previous work (Lee et al., 1995). Many of these volatiles were often selected by other researchers to measure oil oxidation (Neff et al., 1994a; Neff et al., 1994b; Melton et al., 1994). Among these volatile compounds, both *t,c*-2,4-heptadienal and *t,t*-2,4-heptadienal are generally believed to be from the oxidation of 18:3 and both 1-octen-3-ol and 2-octenal from 18:2 (Frankel, 1985).

On day 0, there were no significant differences in amounts of the volatile compounds between 18:3 content groups. Among LOX groups, the only significant differences were in hexenal and 1-octen-3-ol contents. Oils from beans lacking LOX 2 (#2 and #5) had significantly more 1-octen-3-ol and significantly more hexenal, which is a major off-flavor contributor in oxidizing soybean oils, than did oil from normal beans. Soybean oils with normal 18:3 (#1, #2, and #3) tended to have more *t,t*-2,4-heptadienal than did the soybean oils with low 18:3 content (#4, #5, and #6), because this volatile compound was from the oxidation of 18:3. Soybean oils with normal LOX (#1 and #4) tended to have less *t,c*-2,4-heptadienal and *t,t*-2,4-heptadienal than did the other two soybean oils. Soybean oils with normal 18:3 content (#1, #2, and #3) tended to have less 1-octen-3-ol and 2-octenal than did the other oils, because the precursor to these

compounds (18:2) was lower in these oils than in oils with low 18:3 contents (#4, #5, and #6).

On day 16, there were no significant differences between the 18:3 content groups, except for 1-octen-3-ol. The amount of 1-octen-3-ol from the low 18:3 content group (#4, #5, and #6) was significantly greater than that from the normal 18:3 content group (#1, #2, and #3). This observation was likely because of the higher content of 18:2 in oils (#4, #5, and #6). Soybean oils with normal 18:3 content tended to have more *t,c*-2,4-heptadienal than did oils with low 18:3.

Among LOX groups, soybean oils from beans lacking LOX 2 and 3 (#3 and #6) had significantly greater amounts of many of the volatiles than did oils from the other two types. Oils from beans lacking LOX 2 and 3 (#3 and #6) had significantly more 2-pentenal than did oils from beans lacking LOX 2 (#2 and #4). The amounts of 2-heptenal, *t,c*-2,4-heptadienal, and 2-octenal released from soybean oils from seeds missing LOX 2 and 3 (#3 and #6) were greater than that from the other two types. Soybean oils from beans missing LOX 2 and 3 (#3 and #6) released the greatest amount of 1-octen-3-ol, whereas, soybean oils with normal LOX contents (#1 and #4) had the least. In general, the volatile analyses showed that soybean oils from beans missing LOX 2 and 3 (#3 and #6) released more volatile compounds than did the other two groups. On day 16, the PVs confirmed that oils from seeds missing LOX 2 and 3 (#3 and #6) showed greater oxidation than did the other two groups. The calculated oxidizability predicted the same trend as did Study 1. The data in Study 2 reflected the results of Study 1; that soybean oils with low 18:3 contents were significantly more

stable than were oils with normal 18:3 contents. LOX did not play an important role in oxidative stability.

Study 3: Storage of pilot-plant extracted oils at 35 °C under light

Storage of the samples in an oven at 35 °C under fluorescent light oxidized much faster than at 50 or 60 °C in the dark (Table 6 versus Tables 3 and 4). In general, there was a tendency for soybean oils with normal 18:3 contents (#1, #2, and #3) to have the lowest PVs. On days 8 and 14, this tendency was significant. Among LOX groups, the PVs of soybean oils from beans missing LOX 2 and 3 (#3 and #6) were significantly greater than PVs from oils with normal LOX contents from days 8 to 14. The calculated oxidizability correctly predicted greater oxidation of #3 and #6 than that of #1 and #4 (Table 2). The oils from beans missing LOX 2 (#2 and #5) had the greatest overall oxidizability scores, yet tended to be intermediate in PV. Since this study was conducted under light and at 35 °C versus in the dark and at 50 to 60 °C (Study 1 and 2), the oxidation pathway might have been different. The fatty acid compositions of the oils used in Study 3 before and after oxidation are listed in Table 2. Few differences in beginning and ending fatty acid data were noted.

The same volatile compounds as in Study 2 were measured by using the same method (Table 7). On day 0, there were no significant differences in amounts of volatiles between 18:3 content groups and among LOX groups, except for hexenal. Oils from beans missing LOX 2 and 3 (#3 and #6) had significantly less hexenal than did oils from beans missing LOX 2 (#2 and #5). Seeds containing all LOX (#1 and #4) were

intermediate in amount of hexenal. Soybean oils with normal 18:3 content tended to have more *t,c*-2,4-heptadienal and *t,t*-2,4-heptadienal, as expected.

On day 14, there were no significant differences between 18:3 content groups or among LOX groups, and few tendencies were noted. Again, soybean oils with normal 18:3 content tended to have more of both *t,c*-2,4-heptadienal and *t,t*-2,4-heptadienal. Because of different storage conditions, the volatile compounds developed under light were different from those produced in the dark (Evans et al., 1969; Warner et al., 1989).

Sensory evaluations were conducted to determine the sensory quality of fresh and stored samples. Among the oils judged by the sensory panel (Table 8), the oils from the low 18:3 group (#4, #5, and #6) tended to have a lower sensory score (weakest off-flavor) on day 0. By day 14, this tendency was not apparent. Among LOX groups, sensory scores tended to be lowest for oils from beans missing LOX 2 and 3 (#3 and #6) on day 0, and highest for oils from beans missing LOX 2 (#2 and #5). On day 14, oils with normal LOX (#1 and #4) and oils from beans missing LOX 2 and 3 (#3 and #6) were similar, and oils from beans missing LOX 2 (#2 and #5) tended to have the strongest off-flavor. The calculated oxidizability scores were consistent with the high sensory scores for samples #2 and #5. The PVs and volatiles at day 14 did not suggest similar tendencies. Perhaps differences in PV were not great enough for sensory panelists to detect differences in the oils.

The amount of 18:3 is believed to be the main factor contributing to off-flavor development (Hammond, 1992). In the current project, the importance of the amount of 18:3 was shown in Studies 1 and 2 to be the major factor affecting oxidative stability. This phenomenon was not observed during Study 3 where there were no significant differences between 18:3 groups or among LOX groups. Differences in oxidation conditions of light versus dark and of 35 °C versus 50 to 60 °C might have accounted for the different results. The influence of 18:3 content on the oxidative stabilities of soybean oils has not always been observed. Mounts et al. (1988) conducted a study with four soybean genotypes, having 3.3%, 4.2%, 4.8%, or 7.7% 18:3 contents, plus a hydrogenated soybean oil with 3% 18:3 content. After accelerated storage at 60 °C for 4 days, they found no significant differences among the oils in peroxide development during storage.

In this research, soybean oils with low 18:3 contents generally were significantly more stable than were those with normal 18:3 contents, regardless of the LOX content of the beans. The PVs on day 0 in each test indicated the qualities of soybean oils extracted from both laboratory and pilot plant were adequate for these tests. Because storage conditions in Study 3 were different, the oxidation rate was fast, perhaps minimizing differences among the oils. Flavor and oxidative stability of soybean oils may vary depending on the oxidation conditions, such as light versus dark (Evans et al., 1969; Warner et al., 1989).

In general, it seemed that removal of LOX 2 or of LOX 2 and 3 from soybean seeds tended to result in lower oxidative and flavor stability in the oils; however, these differences may have been a result of overall differences in fatty acid compositions, as

also noted from the calculated oxidizability values. All soybean seeds contained at least one LOX. Under this condition, the activities of LOX in the different mutant lines were not known. Furthermore, the time between flaking and extracting could be critical determining the influence of LOX. If the time was too short, there would be no influence of LOX at all. If the time was too long, however, the differences in influences of LOX would be overlooked because oxidation would have reached the maximum rate. The conditions used in this research were designed to mimic the conditions used by the soybean oil industry. Thus, the conclusions obtained could be used to predict the influence of eliminating LOX 2 or LOX 2 and 3 on soybean oil quality of commercially produced oils.

ACKNOWLEDGMENT

The research was partly funded by the Iowa Soybean Promotion Board and by a grant from the United States Department of Agriculture (USDA) for research programs of the Center for Designing Foods to Improve Nutrition. Soybean oil processing and extraction took place in the Center for Crops Utilization Research (CCUR) at Iowa State University. This is Journal paper No. J-xxxx of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Project No. xxxx).

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Table 1. Lipoxygenase and approximate linolenic acid contents of soybean genotypes

Name	Sample Designation	Lipoxygenase (LOX) Content	Approximate Linolenic acid (18:3)
Century 84	#1	normal LOX	>8.0 % 18:3
L2-3	#2	lacking LOX 2	>8.0 % 18:3
L2L3-2-4	#3	lacking LOX 2 and 3	>8.0 % 18:3
A89-269043	#4	normal LOX	<3.0 % 18:3
L2/3%18:3	#5	lacking LOX 2	<3.0 % 18:3
L2L3/3%18:3	#6	lacking LOX 2 and 3	<3.0 % 18:3

Table 2. Fatty acid composition (%) and calculated oxidizability of soybean oils with different lipoxygenase and fatty acid contents before and after storage

		Soybean oil genotypes					
Fatty acid	Status	#1[a]	#2	#3	#4	#5	#6
Study 1							
16:0	start	12.3	13.7	13.3	11.9	12.8	12.4
	end[b]	12.7	13.0	12.9	12.1	13.0	13.0
18:0	start	3.7	3.7	4.1	4.2	3.9	4.1
	end	4.1	4.1	4.2	4.4	4.1	4.2
18:1	start	22.3	19.6	19.9	30.2	22.8	23.5
	end	23.7	21.1	20.5	31.0	23.6	24.0
18:2	start	52.5	53.9	53.7	50.9	57.1	56.5
	end	51.6	53.3	53.5	49.8	56.2	55.5
18:3	start	7.8	8.4	8.4	2.2	2.5	2.5
	end	7.1	7.7	8.1	2.0	2.4	2.4
Oxidizability		7.3	7.6	7.5	6.0	6.6	6.6

[a] See Table 1 for definitions of abbreviations.

[b] Oils were stored for 12 days.

[c] Oils were stored for 22 days.

[d] Oils were stored for 14 days.

Table 2. (Cont.)

Studies 2 and 3							
16:0	start	9.9	10.6	10.7	10.1	11.0	11.2
	Study 2 end[c]	9.8	10.6	10.8	10.1	11.1	11.6
	Study 3 end[d]	9.8	10.6	10.7	10.2	11.2	11.1
18:0	start	4.0	3.8	4.4	4.2	3.9	4.0
	Study 2 end	3.9	3.5	4.2	4.2	3.9	3.7
	Study 3 end	4.5	4.5	4.5	4.8	4.3	4.5
18:1	start	23.1	21.0	21.2	30.9	23.6	23.9
	Study 2 end	23.5	21.5	20.9	31.1	23.6	24.1
	Study 3 end	23.7	21.1	21.3	31.3	23.6	24.2
18:2	start	54.0	55.2	54.7	51.5	57.9	57.3
	Study 2 end	54.1	55.2	55.0	51.3	57.8	57.0
	Study 3 end	53.3	54.7	54.5	50.6	57.4	56.7
18:3	start	8.2	8.6	8.2	2.4	2.8	2.7
	Study 2 end	7.9	8.3	8.3	2.4	2.8	2.7
	Study 3 end	7.9	8.3	8.3	2.4	2.7	2.7
Oxidizability		7.6	7.8	7.6	6.1	6.8	6.7

Table 3. Peroxide values (meq/kg) of laboratory-scale extracted soybean oils during storage at 60 °C in the dark (Study 1)

	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Day	#1[a]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0	0.40	0.15	0.14	0.15	0.14	0.15	0.23[e]	0.15[d]	0.27[d]	0.14[d]	0.14[d]
3	2.24	0.68	0.74	0.68	0.79	0.70	1.22[d]	0.72[d]	1.46[d]	0.73[d]	0.72[d]
6	22.1	17.8	13.0	4.1	5.7	3.2	17.6[e]	4.33[d]	13.1[d]	11.8[d]	8.1[d]
9	70.5	75.4	69.2	46.4	51.6	40.1	71.7[e]	46.0[d]	58.4[d]	63.4[d]	54.6[d]
12	100.2	98.2	106.0	93.3	100.2	106.4	101.5[d]	99.9[d]	96.7[d]	99.4[d]	106.6[d]

[a] See Table 1 for definition of abbreviations.

[b] Within each 18:3 group, M 1-1 = means of PVs of soybean oils with normal 18:3, and M 1-2 = means of PVs of soybean oils with low 18:3.

[c] Within each LOX group, M 2-1 = means of PVs of soybean oils with normal LOX, M 2-2 = means of PVs of soybean oils from beans missing LOX 2, and M 2-3 = means of PVs of soybean oils from beans missing LOX 2 and 3.

[d-e] Values in the same row of each group with different superscripts were significantly different ($P \leq 0.05$).

Table 4. Peroxide values (meq/kg) of pilot-plant extracted soybean oils during storage at 50 °C in the dark (Study 2)

	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Day	#1[a]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0	0.12	0.20	0.13	0.13	0.20	0.12	0.15[d]	0.16[d]	0.13[d]	0.20[d]	0.13[d]
2	0.29	0.37	0.26	0.23	0.30	0.19	0.31[d]	0.25[d]	0.27[de]	0.33[e]	0.23[d]
4	0.43	0.55	0.39	0.35	0.44	0.31	0.47[d]	0.38[d]	0.39[d]	0.49[d]	0.35[d]
6	0.54	0.66	0.47	0.47	0.50	0.40	0.57[d]	0.46[d]	0.50[d]	0.58[d]	0.44[d]
8	0.71	0.92	0.63	0.57	0.62	0.51	0.78[e]	0.58[d]	0.64[de]	0.77[e]	0.57[d]
10	0.82	1.04	0.75	0.65	0.70	0.57	0.89[e]	0.65[d]	0.73[de]	0.87[e]	0.66[d]
12	1.02	1.29	0.99	0.75	0.84	0.75	1.10[e]	0.78[d]	0.87[d]	1.06[d]	0.87[d]

[a] See Table 1 for definitions of abbreviations

[b] Within each 18:3 group, M 1-1 = means of PVs of soybean oils with normal 18:3 and M 1-2 = means of PVs of soybean oils with low 18:3 content.

[c] Within each LOX group, M 2-1 = means of PVs of soybean oils with normal LOX, M 2-2 = means of PVs of soybean oils from beans missing LOX 2, and M 2-3 = means of PVs of soybean oils from beans missing LOX 2 and 3.

[d-e] Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

Table 4. (Cont.)

14	2.15	3.4	3.3	1.07	1.30	1.31	2.89^{e}	1.21^{d}	1.61^{d}	2.35^{d}	2.33^{d}
16	5.6	8.3	9.3	2.20	2.76	4.1	7.41^{e}	2.81^{d}	3.89^{d}	5.52^{d}	6.72^{d}
18	9.6	12.6	15.1	6.0	5.7	9.0	11.9^{e}	5.9^{d}	7.0^{d}	9.1^{d}	12.1^{d}
20	15.1	16.8	23.6	7.6	9.6	14.8	17.5^{e}	9.8^{d}	11.3^{d}	13.2^{de}	19.2^{e}
22	23.8	23.9	33.2	11.8	15.1	21.6	25.7^{e}	15.1^{d}	17.8^{d}	19.5^{de}	27.4^{e}

Table 5. Peak areas (10^{-5})of volatile compounds from pilot-plant extracted soybean oils stored at 50 °C in the dark (Study 2)

Volatile	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Compound	#1[a]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
Day 0											
Pentanal	0.31	0.11	0.60	0.03	0.50	0.20	0.39[d]	0.44[d]	0.17[d]	0.34[d]	0.74[d]
2-Pentenal	0.10	0.25	1.41	0.09	0.14	0.00	0.58[d]	0.10[d]	0.09[d]	0.19[d]	0.74[d]
Hexenal	0.39	2.10	1.00	1.03	3.1	1.68	1.42[d]	1.93[d]	0.71[d]	2.60[e]	1.72[de]
2-Heptenal	0.08	1.38	1.28	0.13	4.1	0.49	0.91[d]	1.78[d]	0.10[d]	3.04[d]	0.88[d]
1-Octen-3-ol	0.09	0.33	0.28	0.00	1.02	0.20	0.23[d]	0.41[d]	0.04[d]	0.67[e]	0.24[de]
t,c-2,4-Heptadienal	0.00	0.45	0.73	0.15	0.53	0.54	0.39[d]	0.40[d]	0.07[d]	0.48[d]	0.63[d]

[a] See Table 1 for definitions of abbreviations.

[b] Within each 18:3 group, M 1-1 = means of volatile compounds of soybean oils with normal 18:3 content and M 1-2 = means of volatile compounds of soybean oils with low 18:3 content.

[c] Within each LOX group, M 2-1 = means of volatile compounds of soybean oils with normal LOX content, M 2-2 = means of volatile compounds of soybean oils from beans missing LOX 2, and M 2-3 = means of volatile compounds of soybean oils from beans missing LOX 2 and 3.

[d-f] Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

Table 5. (Cont.)

t,t-2,4-Heptadienal	0.29	1.16	2.60	0.42	2.01	0.55	1.37^{d}	0.99^{d}	0.35^{d}	1.58^{d}	1.61^{d}
2-Octenal	0.33	1.22	0.92	0.30	1.80	0.85	0.82^{d}	1.12^{d}	0.53^{d}	1.51^{d}	0.88^{d}
Nonanal	0.90	1.80	2.86	0.93	2.08	2.01	1.81^{d}	1.66^{d}	0.84^{d}	1.94^{d}	2.43^{d}
Day 16											
Pentanal	0.26	0.20	0.35	0.22	0.31	0.38	0.30^{d}	0.30^{d}	0.24^{d}	0.29^{d}	0.36^{d}
2-Pentenal	1.20	0.81	3.0	0.63	1.11	2.00	1.85^{d}	1.27^{d}	1.17^{de}	0.96^{d}	2.54^{e}
Hexenal	0.80	0.85	16.1	8.2	22.0	19.2	10.6^{d}	16.7^{d}	8.0^{d}	15.3^{d}	17.6^{d}
2-Heptenal	16.1	0.58	4.0	18.0	38.4	50.9	23.8^{d}	35.8^{d}	17.0^{d}	23.0^{d}	49.3^{e}
1-Octen-3-ol	5.0	3.1	9.7	5.7	23.9	35.4	5.9^{d}	21.7^{e}	5.3^{d}	13.5^{e}	22.6^{f}
t,c-2,4-Heptadienal	5.0	1.15	34.0	0.63	4.8	6.9	13.6^{d}	6.5^{d}	6.3^{d}	3.0^{d}	20.8^{e}
t,t-2,4-Heptadienal	4.4	1.45	14.0	5.1	8.4	6.1	6.6^{d}	6.5^{d}	4.8^{d}	4.9^{d}	10.0^{d}
2-Octenal	3.0	1.01	8.4	5.2	5.1	6.8	4.1^{d}	5.7^{d}	4.1^{d}	3.0^{d}	7.6^{e}
Nonanal	1.84	0.60	2.41	2.5	2.9	1.00	1.67^{d}	2.20^{d}	2.17^{d}	1.82^{d}	1.81^{d}

Table 6. Peroxide values (meq/kg) of pilot-plant extracted soybean oils during storage at 35 °C under light (Study 3)

	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Day	#1[a]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0	0.26	0.34	0.34	0.30	0.36	0.28	0.31[d]	0.31[d]	0.28[d]	0.35[d]	0.31[d]
2	1.53	1.71	1.99	1.54	1.69	1.65	1.74[d]	1.63[d]	1.54[d]	1.70[d]	1.8[d]
4	2.45	2.73	4.8	3.5	3.4	5.7	3.22[d]	4.2[d]	2.98[d]	3.1[d]	5.1[d]
6	4.2	5.1	8.2	7.2	7.3	12.6	5.8[d]	9.0[d]	5.7[d]	6.2[d]	10.4[d]
8	6.3	9.8	15.8	12.7	14.8	21.9	10.6[d]	16.4[e]	9.5[d]	12.3[d]	18.8[e]
10	14.3	17.4	21.8	19.2	22.7	29.6	17.8[d]	23.8[d]	16.7[d]	20.0[de]	25.7[e]
12	19.6	22.1	30.1	26.1	27.5	35.5	23.9[d]	29.7[d]	22.8[d]	24.8[de]	32.8[e]
14	25.2	28.8	34.5	31.1	34.4	41.2	29.5[d]	35.5[e]	28.1[d]	31.6[de]	37.8[e]

[a] See Table 1 for definitions of abbreviations.

[b] Within each 18:3 group, M 1-1 = means of PVs of soybean oils with normal 18:3 and M 1-2 = means of PVs of soybean oils with low 18:3 content.

[c] Within each LOX group, M 2-1 = means of PVs of soybean oils with normal LOX, M 2-2 = means of PVs of soybean oils from beans missing LOX 2, and M 2-3 = means of PVs of soybean oils from beans missing LOX 2 and 3.

[d-e] Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

Table 7. Peak areas (10^{-5})of volatile compounds of pilot-plant extracted soybean oils stored at 35 °C under light (Study 3)

Volatile	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Compound	#1[a]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
Day 0											
Pentanal	1.45	0.68	5.85	0.30	0.38	0.20	2.66[d]	0.29[d]	0.88[d]	0.53[d]	3.03[d]
2-Pentenal	0.50	0.25	0.63	0.10	0.55	0.13	0.46[d]	0.26[d]	0.30[d]	0.40[d]	0.38[d]
Hexenal	2.65	2.25	2.23	1.43	5.15	1.13	2.38[d]	2.57[d]	2.04[de]	3.70[e]	1.68[d]
2-Heptenal	0.65	0.80	1.30	0.20	1.50	0.48	0.91[d]	0.81[d]	0.43[d]	1.26[d]	0.89[d]
1-Octen-3-ol	0.45	0.93	0.90	4.9	8.7	3.60	5.4[d]	5.7[d]	6.2[d]	4.8[d]	5.8[d]
t,c-2,4-Heptadienal	1.83	1.10	1.20	0.48	2.38	0.33	1.38[d]	1.06[d]	1.15[d]	1.74[d]	0.76[d]

[a] See Table 1 for definitions of abbreviations.

[b] Within each 18:3 group, M 1-1 = means of volatile compounds of soybean oils with normal 18:3 content and M 1-2 = means of volatile compounds of soybean oils with low 18:3 content.

[c] Within each LOX group, M 2-1 = means of volatile compounds of soybean oils with normal LOX content, M 2-2 = means of volatile compounds of soybean oils from beans missing LOX 2, and M 2-3 = means of volatile compounds of soybean oils from beans missing LOX 2 and 3.

[d-e] Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

Table 7. (Cont.)

t,t-2,4-Heptadienal	1.13	1.50	1.83	0.98	0.83	0.95	1.48[d]	0.92[d]	1.05[d]	1.16[d]	1.39[d]
2-Octenal	3.25	1.48	3.68	2.40	1.95	1.63	2.80[d]	1.99[d]	2.83[d]	1.71[d]	2.65[d]
Nonanal	2.23	1.58	2.28	0.00	1.50	0.60	2.03[d]	0.93[d]	1.46[d]	1.54[d]	1.44[d]
Day 14											
Pentanal	2.13	2.13	1.60	1.30	2.43	1.33	1.95[d]	1.68[d]	1.71[d]	2.28[d]	1.46[d]
2-Pentenal	3.20	5.5	11.9	5.2	6.4	11.0	6.9[d]	7.7[d]	4.2[d]	6.0[d]	11.8[d]
Hexenal	52.1	61.9	5.2	4.8	62.0	6.5	57.0[d]	68.1[d]	63.5[d]	61.9[d]	62.3[d]
2-Heptenal	53.5	59.0	51.3	66.9	59.0	50.6	54.6[d]	59.1[d]	60.2[d]	59.3[d]	51.0[d]
1-Octen-3-ol	38.1	2.40	20.5	43.4	38.0	30.4	28.6[d]	37.5[d]	40.7[d]	33.0[d]	25.4[d]
t,c-2,4-Heptadienal	2.15	3.3	3.8	1.65	2.18	1.88	3.11[d]	1.90[d]	1.90[d]	2.75[d]	2.86[d]
t,t-2,4-Heptadienal	6.5	2.80	3.8	0.50	2.60	1.00	4.4[d]	1.68[d]	3.60[d]	2.69[d]	2.76[d]
2-Octenal	2.33	2.38	3.2	3.2	3.3	4.0	2.63[d]	3.49[d]	2.78[d]	2.84[d]	3.56[d]
Nonanal	3.2	1.25	1.50	0.80	0.65	1.03	2.05[d]	0.82[d]	1.96[d]	0.95[d]	1.39[d]

Table 8. Sensory evaluation scores (cm)[a] of pilot-plant extracted soybean oils stored at 35 °C under light (Study 3)

	Soybean oil genotypes						18:3 group[c]		LOX group[d]		
Day	#1[b]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
day 0	5.0	4.5	3.8	3.0	5.9	3.0	4.4[e]	3.6[e]	4.0[e]	5.0[e]	3.4[e]
day 14	8.1	8.6	8.3	7.9	10.7	7.8	8.3[e]	8.4[e]	8.0[e]	9.3[e]	8.0[e]

[a] A score of 1 = weakest and 15 = strongest off-flavor.

[b] See Table 1 for definitions of abbreviations.

[c] Within each 18:3 group, M 1-1 = means of sensory scores of soybean oils with normal 18:3 and M 1-2 = means of sensory scores of soybean oils with low 18:3 content.

[d] Within each LOX group, M 2-1 = means of sensory scores of soybean oils with normal LOX, M 2-2 = means of sensory scores of soybean oils from beans missing LOX 2, and M 2-3 = means of sensory scores of soybean oils from beans missing LOX 2 and 3.

[e] Values in the same row within each group with different superscripts were significantly different ($P \leq 0.05$).

HIGH-TEMPERATURE STABILITIES OF SOYBEAN OILS LACKING LIPOXYGENASES

A paper to be submitted to the Journal of the American Oil Chemists' Society

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ABSTRACT

Lipoxygenase (LOX)-null soybean lines lacking LOX 2, or LOX 2 and 3 and containing normal (8.0 - 8.6%) or low (2.0 - 2.8%) linolenate (18:3) amounts were evaluated for their stability during frying and for oxidative stability in bread cubes stored after frying. Soybean oils were extracted by a pilot-plant scale system and were refined, bleached, and deodorized in the laboratory. Citric acid was added to oils during the cool-down stage of deodorization. Two replications separated at the point of conditioning were evaluated for each genotype. Each replicate (250 g) was heated to 180 ± 5 °C in a minifryer. Bread cubes were fried at the beginning of heating and after 20 h of heating. Heating of the oils was continued for 10 h each day for 3 consecutive days. Soybean oils with low 18:3 contents were significantly more stable as measured by conjugated dienoic acids and polymer values than were oils with normal 18:3 contents, regardless of the LOX contents of the beans. Peroxide values of soybean oils extracted from bread cubes tended to give the same conclusion. The sensory evaluation, however, did not differentiate

between oils containing low or high 18:3 amounts or among oils from beans lacking different LOX enzymes.

KEY WORDS: conjugated dienoic acids, fatty acids, oxidative stability, polymerization, and soybean oils.

INTRODUCTION

The flavor instability of soybean oil and the tendency of soybean oil to polymerize limits its use in frying temperature used for food preparation. The main cause of the flavor instability is the linolenate (18:3) content of approximately 8% in normal soybean oil. Other vegetable oils, such as corn, sunflower, and cottonseed have less than 1.0% 18:3 and are adequate for cooking or frying use (Huang et al., 1981). A common way to lower the 18:3 content in soybean oil is partial hydrogenation. Keijbets et al. (1985) reported that hydrogenated soybean oil with reduced 18:3 content was more stable than unhydrogenated oil in prefrying of deep-frozen products.

Mensink and Katan (1990) reported that *trans* fatty acids (*t*FAs) formed during hydrogenation increased low-density lipoprotein cholesterol and lower high-density lipoprotein in human plasma. Hydrogenated soybean oil is claimed to be the main source of *t*FAs in U.S. diet (Gurr, 1983). Mag (1994) suggested that margarine consumption in Canada has declined at least partly because of *t*FAs.

To avoid *t*FA and improve stability of soybean oil, the 18:3 content of soybeans has been reduced genetically to 3% (Hammond and Fehr, 1984). Soybean oil with

reduced 18:3 (<3%) was found to be more stable at frying temperatures than that with normal 18:3 (8%) (Miller and White, 1988; Liu and White, 1992). Mounts et al. (1994) noted that low-18:3 soybean oils could be alternative frying oils. Warner et al. (1994) reported that the best flavor stability of potato chips was obtained from a frying oil with 68% oleic acid, 20% linoleic acid, and 3% 18:3. Recently, Kraft Food Ingredients began to market a low 18:3 (3%) soybean oil, named SOY•LL. The soybean cultivars with reduced 18:3 used for SOY•LL were developed at Iowa State University and grown by Pioneer Hi-Bred International Inc. (Anonymous, 1994).

Lipoxygenase (LOX) enzymes are believed to contribute to flavor instability (Davies and Nielsen, 1986; Frankel et al., 1988; White, 1992; Kitamura, 1995). Genetic removal of LOX 2 reduced off-flavors in soy foods, such as soy milk (Davies et al., 1987); however, Frankel et al. (1988) found that the absence of LOX 1 did not affect the oxidative stability of soybean oil. Endo et al. (1990) studied the oxidative stability of soybean oil from LOX 1- and 3-null and LOX 2- and 3-null beans. The oils were stored at 30 °C under light. Sensory evaluation and peroxide values were not affected by combinations of LOX on oxidative stability.

The objective of this study was to evaluate oil from soybeans with normal and reduced LOX contents in combination with normal and reduced 18:3 for their high temperature stabilities and oxidative stabilities of bread cubes stored after frying.

MATERIALS AND METHODS

Century 84, L2-3, and L2L3-2-4 were developed by the USDA-ARS and Purdue University. A89-269043, A89-269043-L2, and A89-269043-L2L3 were developed at Iowa State University in 1992 (Table 1).

A89-269043-L2 and A89-269043-L2L3 were developed from the cross L2L3-2-4 x A89-269043. The third filial generation (F3) derived lines that contained <3% 18:3 and that lacked LOX 2 were composited to form A89-269043-L2. A89-269043-L2L3 was a composite of F3-derived lines with <3% 18:3 and that lacked LOX 2 and 3.

Soybean seeds of six genotypes (Table 1) were processed and analyzed as described by Shen et al. (submitted). Two replicates of 250 g of the oil were used for deep-fat frying.

Each replicate was placed into a teflon-coated electric minifryer with a total capacity of 473 mL (Presto Fry Baby Electric Fryer, National Presto Industries, Inc., Eau Claire, WI) and heated to 180 ± 5 °C within 10 min. One batch (40 g) of 1.0 $inch^3$ crust-free bread cubes was fried for 1.5 min and drained. Part of the fried bread cubes was loosely covered and stored at 60 °C in the dark for 4 days. The rest of the cubes were extracted with hexane and the resulting oil was analyzed for peroxide value.

Soybean oils were heated 10 h for 3 consecutive days with cooling to room temperature overnight. At the end of each day of heating, a 20-g oil sample was removed and stored under nitrogen at -14 °C until analyzed for fatty acid composition, conjugated dienoic acid value, and polymer value. The oil was not replenished or filtered. After 20 h of heating, additional bread cubes (40 g) were fried for 1.5 min and

stored for sensory evaluation and peroxide value determination. Bread cubes were loosely covered and stored at 60 °C in the dark for four days.

Additional bread cubes were fried in fresh Wesson® vegetable oil (100 % soybean oil) that was purchased from a local grocery store. These cubes were stored either fresh or aged at -14 °C in freezer bags with the air pressed out by hand and were used as references during sensory training and evaluation.

Peroxide values of oil from fried bread cubes The peroxide values (PV) of the oils extracted from the cubes were determined by using the Stamm test as modified by Hamm et al. (1965). The oil was extracted from bread cubes (3 g) with 25 mL hexane three times for 10 min each. The solvent was removed by rotary evaporation (Miller and White, 1988).

Sensory evaluation Eleven trained panelists evaluated the fried bread cubes at room temperature for intensity of oxidized flavor according to the AOCS official method Cg 2-83 (AOCS, 1989). On the flavor intensity scale, 10 is bland and 1 is an extremely intense flavor. The panelists were trained by judging fresh and stored bread cubes fried in Wesson vegetable oil. Three training sessions were conducted to develop agreement on oxidized flavor scores. The fried cubes were presented at room temperature and in two sets based on 18:3 contents. Set 1 included soybean oils with normal 18:3 contents and set 2 with low 18:3 contents. In each set, there were three samples, one had normal LOX content (#1 or #4), one was from seeds lacking LOX 2 (#2 or #5), and one was from beans lacking LOX 2 and 3 (#3 or #6).

The panelists were instructed to first smell the cubes and then to taste them in an approximate order of increasing odor intensity. This procedure reduced the possibility of a strongly oxidized sample overwhelming a panelist's ability to evaluate less oxidized samples before all samples were judged. Panelists were instructed to expectorate after chewing and evaluating each sample. Distilled water and unsalted crackers were provided to rinse the oxidized flavor from their mouths. The thawed fried cubes that had been fried in Wesson vegetable oil and stored at -14 °C were provided as a reference in judging the samples (Liu and White, 1992).

Fatty acid compositions Fatty acid compositions were measured at 0 h, 10 h, 20 h, and 30 h of heating. Fatty acid methyl esters (FAMES) of the frying oils were prepared by transesterifying the oils with sodium methoxide in methanol and injecting in a GC, as described by Hammond (1991). The theoretical response factors for each fatty acid were applied as calculated by Ackman (1992).

Conjugated dienoic acid The conjugated dienoic acids (CDA) were measured to estimate shifting of double bonds during oxidation according to AOCS official method Ti 1a-64 (AOCS, 1989). Soybean oils (0.100 g) were dissolved in 25 mL of isooctane, diluted, and the absorbency was measured at 233 nm.

High-performance size-exclusion chromatography High-performance size-exclusion chromatography (HPSEC) was used to measure polymerized high-molecular weight compounds formed during heating (White and Wang, 1986). The

HPSEC system was composed of a Beckman 110A pump, 20-μL injector loop, Beckman 210 sample injector (Beckman Instruments, Inc., Fullerton, CA), Hitachi 100-10 variable wavelength UV/VIS detector (Hitachi, Ltd., Tokyo, Japan), a Beckman 10-inch strip-chart recorder and two [500 Å (0.8 x 30 cm) and 1000 Å (0.77 x 30 cm)] μ-Spherogel columns (Altex Scientific, Inc., Berkeley, CA). A column inlet filter between the injector and the column was used to prevent blockage of the column inlet frit. HPLC-grade methylene chloride (Fisher Scientific, Pittsburgh, PA) was used as the mobile phase, with a flow rate of 1 mL/min. The sample preparation involved dissolving 0.600 g ± 0.005 g of a heated oil in 4.5 mL of HPLC-grade methylene chloride. The UV wavelength of 254 nm was used for measurement.

Polystyrene standards (Supelco, Inc., Bellefonte, PA) of various molecular weights (794, 2,000, 2,500, 5,000, 9,000, 17,500, 30,000, and 50,000 g/mole) were used as external standards to determine the approximate molecular weight separation on the columns.

Statistical analysis A randomized 2 x 3 factorial design was used for analysis of the data. Data from all treatments in each test were analyzed by using ANOVA and LSD for statistical significance (SAS, 1985).

RESULTS AND DISCUSSION

Frying oils Polyunsaturated fatty acids decreased in amount during heating, especially 18:3 (Table 2). After 30 h of heating, the 18:3 content of #6 declined to 1.0%,

which was only 40% of the original amount. Other soybean oils lost about 50% of their 18:3 contents. When 18:3 content was reduced, the relative percentage of saturated fatty acids increased (over 50% for 16:0 and 18:0). This pattern also was observed in other studies (Miller and White, 1988; Liu and White, 1992).

There was a tendency for low 18:3 oils (#4, #5, and #6) to have lower CDA values than normal 18:3 oils (#1, #2, and #3) throughout the heating period (Table 3). After 0 and 30 h of heating, the differences were significant. Other researchers also noted less CDA formation in low 18:3 oils (Miller and White, 1988; Liu and White, 1992). Soybean oils with normal LOX contents tended to have lower CDA values than did the other two oils in the LOX group. This finding was significant at 30 h of heating. During heating, autoxidation of polyunsaturated fatty acids causes a shift in the double bonds producing a conjugated diene that can be measured by ultraviolet absorption at 233 nm (Gray, 1985; White, 1991). Absorption at this wavelength increases proportionally to oxidation in the early stages but plateaus during frying because of the establishment of an equilibrium between the rate of formation of conjugated dienes and the rate of polymerization (White, 1991).

HPSEC of the oils showed differences in formation of high-molecular weight (MW) compounds of the oils formed during frying. The standard curve for retention times of polystyrene standards is presented in Figure 1. There were 4 peaks formed during heating of the oils (White and Wang, 1986). Peak 1 represented triacylglycerides and fatty acid trimers with MW of 1,000 g/mole, peak 2 represented dimeric triglycerides with MW of 2,000 g/mole,, peak 3 represented tetrameric triacylglycerides with MW of 4,000 g/mole, and peak 4 represented tetrameric

triacylglycerides with MW of >4,000 g/mole. In our study, however, only three peaks were measurable. Although present, peak 3 was not measurable.

The high MW compounds, represented as peaks 2 and 4, formed in the greatest quantities during heating (Table 4). When the oils were heated to 180 °C, soybean oils with normal 18:3 content (#1, #2, and #3) tended to have greater amounts of all peaks than did oils with low 18:3 content (#4, #5, and #6). Some differences were significant. The data from HPSEC had a similar pattern to that of CDA values. In both cases, soybean oils with normal 18:3 contents were not as stable as soybean oils with low 18:3 content.

There were no significant differences and no notable trends within the LOX group (#1 and #4, #2 and #5, of #3 and #6) throughout the study, except for 0 h of heating, when soybean oils with normal LOX (#1 and #4) had a significantly greater amount of peak 2 than did the other two groups of oils within the LOX group (#2 and #5; and #3 and #6) (Table 4).

Bread cubes The PVs of soybean oils extracted from bread cubes fried at 0 h without storage were similar to each other (Table 5). After storage for 4 days, PVs of oils from bread cubes fried in low 18:3-oils tended to be lower than from oils with normal 18:3 content. The PVs of soybean oils from beans lacking LOX 2 and 3 (#3 and #6) tended to be the highest among the LOX group, but the differences were not significant. For oils from bread cubes fried after 20 h of heating, PVs from normal-18:3 content oils tended to be higher than those from low-18:3 content oils, regardless of the time of frying. After 4 days of storage, PVs of oils from bread cubes fried in soybean oils

from beans lacking LOX 2 tended to be the greatest among the LOX group, whereas PVs of soybean oils with normal LOX content tended to be the lowest.

Sensory evaluations were done on the stored bread cubes (Table 6). When bread cubes were fried at 0 h and stored in the dark at 60 °C for 4 days, cubes fried in soybean oils with normal 18:3 contents tended to have better sensory scores (less off-flavor). When bread cubes were fried in oils after 20 h of heating and stored for 4 days, those fried in soybean oils with normal 18:3 content tended to have slightly more off-flavor than cubes fried in soybean oils with low 18:3 contents. Bread cubes fried in soybean oils with normal LOX contents tended to have the best scores (the least off-flavor) among the LOX group for both types of stored bread cubes. The sensory data for bread cubes fried at 20 h had the same tendency as did the PVs. The calculated oxidizability also showed that soybean oils with low 18:3 contents were more stable than were those with normal 18:3 content.

This study showed that soybean oils with low 18:3 contents had significantly better frying stabilities than did oils with normal 18:3 contents. The results are similar to a previous study that evaluated the accelerated room temperature storage of these same oils (Shen et al., submitted). In general, removal of LOX did not affect the frying stability of soybean oils. Because the activity of LOX 1 in soybean mutant lines was not known, its influence on the frying stability of soybean oils could not be determined. Furthermore, the time between flaking and extracting could be critical determining the influence of LOX. If the time was too short, there would be no influence of LOX at all. If the time was too long, however, the differences in influences of LOX would be overlooked because oxidation would have reached the maximum rate. The oil

extraction procedure used in this research mimicked that of commercial oil extractions, which may have influenced the effects of LOX on frying stability of soybean oils. At least, the conclusion obtained could be used to predict the influence of LOX on the frying stability of soybean oil in soybean oil industry.

ACKNOWLEDGMENT

The research was partly funded by the Iowa Soybean Promotion Board and by a grant from the United States Department of Agriculture (USDA) for research programs of the Center for Designing Foods to Improve Nutrition. Soybean oil processing and extraction took place in the Center for Crops Utilization Research (CCUR) at Iowa State University. This is Journal Paper No. J-xxxxx of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Project No. xxxx).

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Table 1. Lipoxygenase and approximate linolenic acid contents of soybean genotypes used for the experiment.

Name	Abbreviation	Lipoxygenase (LOX) content	Approximate 18:3 content
Century 84	#1	normal LOX	>8.0% 18:3
L2-3	#2	lacking LOX 2	>8.0% 18:3
L2L3-2-4	#3	lacking LOX 2 and 3	>8.0% 18:3
A89-269043	#4	normal LOX	<3.0% 18:3
A89-269043-L2	#5	lacking LOX 2	<3.0% 18:3
A89-269043-L2L3	#6	lacking LOX 2 and 3	<3.0% 18:3

Table 2. Mean fatty acid composition (%) and calculated oxidizability of soybean oils at different heating times.

	Heating	Soybean oil genotypes					
Fatty acid	h	#1[a]	#2	#3	#4	#5	#6
16:0	0	10.2	11.1	11.1	10.7	11.5	11.6
	10	11.2	12.3	12.8	11.7	12.4	12.3
	20	12.2	13.3	13.4	12.1	13.9	13.4
	30	15.8	17.7	18.0	15.8	17.1	17.5
18:0	0	4.0	4.1	4.0	4.2	3.8	4.0
	10	4.2	4.2	4.3	4.4	4.1	4.1
	20	4.7	4.8	4.9	4.9	4.4	4.6
	30	5.8	6.2	6.3	5.8	5.7	5.5
18:1	0	22.7	20.6	19.7	30.1	22.6	23.0
	10	23.4	20.6	20.2	30.7	23.3	23.9
	20	25.9	23.2	22.9	33.4	25.1	26.0
	30	29.7	27.6	27.1	37.2	29.9	29.1

[a] Abbreviations in Table 1.

[b] Calculated oxidizability = [oleate% + 10.3 (linoleate%) + 21.6 (linoleneate%)]/100 (Fatemi and Hammond, 1980)

Table 2. (Cont.)

18:2	0	54.0	54.9	55.5	51.7	58.3	57.7
	10	52.6	53.8	53.8	50.0	56.6	56.1
	20	50.1	51.3	51.5	47.1	53.6	53.3
	30	43.8	43.9	44.1	39.7	45.3	45.9
18:3	0	8.2	8.5	8.8	2.4	2.9	2.9
	10	7.7	8.2	8.1	2.4	2.7	2.7
	20	6.3	6.6	6.6	1.9	2.4	2.2
	30	4.3	4.1	4.0	1.0	1.5	1.6
Oxidizability[b]		7.6	7.7	7.8	6.1	6.9	6.8

Table 3. Conjugated dienoic acid values (%) of soybean oils heated at 180 °C.

Heating	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
h	#1[a]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0	0.34	0.35	0.38	0.26	0.25	0.21	0.36[e]	0.24[d]	0.30[d]	0.30[d]	0.30[d]
10	1.63	1.67	1.73	1.40	1.47	1.49	1.68[d]	1.45[d]	1.51[d]	1.57[d]	1.61[d]
20	3.19	3.27	3.25	2.65	2.79	2.78	3.24[d]	2.74[d]	2.92[d]	3.03[d]	3.01[d]
30	4.07	4.20	4.20	3.60	4.02	4.02	4.16[e]	3.88[d]	3.83[d]	4.11[e]	4.11[e]

[a] Designation of Abbreviations in Table 1.

[b] Within each 18:3 group, M 1-1 = means of conjugated dienoic acid values of soybean oils with normal 18:3 content and M 1-2 = means of conjugated dienoic acid values of soybean oils with low 18:3 content.

[c] Within each LOX group, M 2-1 = means of conjugated dienoic acid values of soybean oils with normal LOX content, M 2-2 = means of conjugated dienoic acid values of soybean oils from beans missing LOX 2, and M 2-3 = means of conjugated dienoic acid values of soybean oils from beans missing LOX 2 and 3.

[d-e] Values in the same row within each category with different superscripts were significantly different ($P \leq 0.05$).

Table 4. Peak areas (cm^2) from HPSEC analysis of soybean oils heated to 180 °C

		Soybean oil genotypes						18:3 group[b]		LOX group[c]		
Time	Peak	#1[a]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0	1	4.4	4.3	4.6	3.4	3.3	2.58	4.43[e]	3.08[d]	3.89[d]	3.80[d]	3.58[d]
	2	0.54	0.37	0.31	0.27	0.25	0.19	0.40[e]	0.24[d]	0.40[e]	0.31[d]	0.25[d]
10	1	7.4	7.7	7.7	6.5	6.3	5.8	7.6[e]	6.2[d]	7.0[d]	7.0[d]	6.8[d]
	2	10.2	10.1	9.9	9.0	9.3	9.8	10.1[d]	9.3[d]	9.6[d]	9.7[d]	9.8[d]
20	1	11.2	12.2	12.4	10.1	11.2	9.7	12.0[d]	10.3[d]	10.7[d]	11.7[d]	11.1[d]
	2	23.3	22.7	23.0	19.5	20.7	20.7	23.0[d]	20.3[d]	21.4[d]	21.7[d]	21.8[d]
	4	2.14	1.95	1.72	1.40	1.42	1.14	1.9[d]	1.3[d]	1.8[d]	1.7[d]	1.4[d]
30	1	11.9	11.9	12.2	11.6	11.9	11.6	12.0[d]	11.7[d]	11.7[d]	11.9[d]	11.9[d]
	2	20.8	19.5	21.4	23.4	25.9	26.5	20.6[d]	25.3[d]	22.1[d]	22.7[d]	23.9[d]
	4	17.0	19.3	17.6	9.6	8.6	7.9	18.0[e]	8.7[d]	13.3[d]	13.9[d]	12.8[d]

[a] See Table 1 for definitions of abbreviations.

[b] Within each 18:3 group, M 1-1 = means of peak areas of soybean oils with normal 18:3 and M 1-2 = means of peak areas of soybean oils with low 18:3 content.

[c] Within each LOX group, M 2-1 = means of peak areas of soybean oils with normal LOX, M 2-2 = means of peak areas of soybean oils from beans missing LOX 2, and M 2-3 = means of peak areas of soybean oils from beans missing LOX 2 and 3.

[d-e] Values in the same row within each category with different superscripts were significantly different ($P \leq 0.05$).

Table 5. Peroxide values (meq/kg) of soybean oils extracted from bread cubes before and after storage at 60 °C in the dark

	Soybean oil genotypes						18:3 group[b]		LOX group[c]		
	#1[a]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
Bread cubes fried at 0 h of heating and stored											
0 day	1.3	1.5	1.2	1.4	1.4	1.4	1.3[d]	1.4[d]	1.3[d]	1.4[d]	1.3[d]
4 days	27.1	25.8	37.7	10.1	11.5	14.5	30.2[d]	12.0[d]	18.6[d]	18.7[d]	26.1[d]
Bread cubes fried after 20 h of heating and stored											
0 day	5.6	5.5	7.2	4.0	3.8	4.3	6.1[d]	4.1[d]	4.8[d]	4.7[d]	5.7[d]
4 days	304.2	510.8	452.1	202.1	399.0	251.0	422.4[d]	284.1[d]	253.2[d]	454.9[d]	351.6[d]

[a] See Table 1 for definitions of abbreviations.

[b] Within each 18:3 group, M 1-1 = means of PVs of soybean oils with normal 18:3 and M 1-2 = means of PVs of soybean oils with low 18:3 content.

[c] Within each LOX group, M 2-1 = means of PVs of soybean oils with normal LOX, M 2-2 = means of PVs of soybean oils from beans missing LOX 2, and M 2-3 = means of PVs of soybean oils from beans missing LOX 2 and 3.

[d] Values in the same row within each category with different superscripts were significantly different ($P \leq 0.05$).

Table 6. Flavor intensity scores[a] of bread cubes fried in soybean oils (180 °C) at 0 h and 20 h of heating and stored at 60 °C for 4 days in the dark

	Soybean oil genotypes						18:3 group[c]		LOX group[d]		
Heating	#1[b]	#2	#3	#4	#5	#6	M1-1	M1-2	M2-1	M2-2	M2-3
0 h	7.3	7.6	7.0	7.4	6.2	6.3	7.3[e]	6.6[e]	7.4[e]	6.9[e]	6.6[e]
20 h	5.7	5.2	4.6	6.2	4.5	5.4	5.1[e]	5.4[e]	5.9[e]	4.8[e]	5.0[e]

[a] A score of 1 = extreme and 10 = bland off-flavor.

[b] See Table 1 for definitions of abbreviations.

[c] Within each 18:3 group, M 1-1 = means of sensory scores of soybean oils with normal 18:3 and M 1-2 = means of sensory scores of soybean oils with low 18:3 content.

[d] Within each LOX group, M 2-1 = means of sensory scores of soybean oils with normal LOX, M 2-2 = means of sensory scores of soybean oils from beans missing LOX 2, and M 2-3 = means of sensory scores of soybean oils from beans missing LOX 2 and 3.

[e] Values in the same row within each category with different superscripts were significantly different ($P \leq 0.05$).

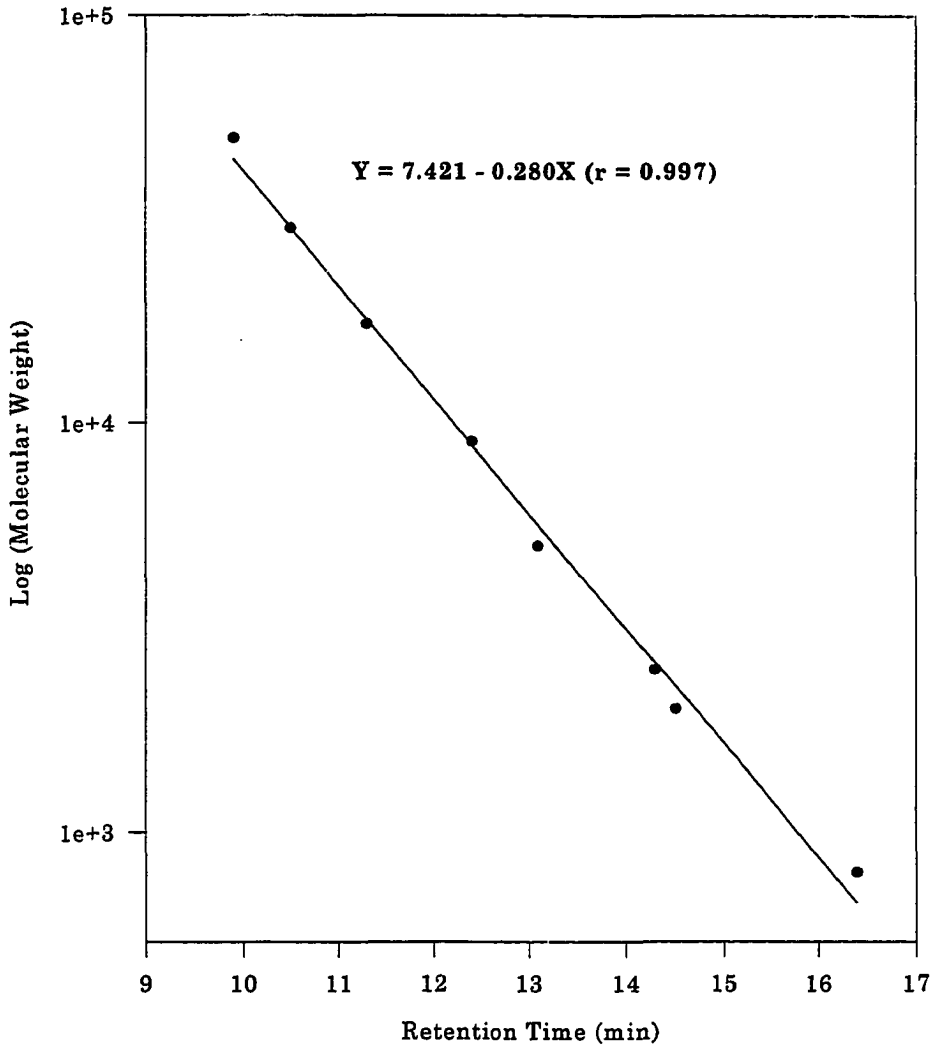

Figure 1. Retention time of polystyrene standards compared with log of their molecular weight

GENERAL CONCLUSIONS

Lipoxygenase (LOX)-null soybean lines, lacking LOX 2, LOX 2 and 3 and containing normal (8.0 - 8.6%) or low (2.0 - 2.8%) amounts of linolenate (18:3) were evaluated for their room-temperature oxidative stabilities, stabilities during frying, and oxidative stability in bread cubes stored after frying. Samples tested included Century 84 (> 8% 18:3 and not lacking LOX), L2-3 (>8% 18:3 and lacking LOX 2), L2L3-2-4 (>8% 18:3 and lacking LOX 2 and 3), A89-269043 (<3% 18:3 and not lacking LOX), A89-269043-L2 (<3% 18:3 and lacking LOX 2), and A89-269043-L2L3 (<3% 18:3 and lacking LOX 2 and 3).

In storage studies, peroxide values, fatty acid compositions, volatile compounds, and sensory evaluations were measured. Soybean oils with low 18:3 contents were found to be significantly more stable than oils with normal 18:3 contents, regardless of the LOX contents of the beans. When the samples were tested at frying temperature (180 °C), sensory evaluation of the fried bread cubes, the peroxide values of oils extracted from the bread cubes, and the conjugated dienoic acid values and polymer values of the heated oils were determined. Soybean oils with low 18:3 contents were significantly more stable than were oils with normal 18:3 contents, regardless of the LOX contents of the beans. Soybean oils containing different amounts of palmitate (16:0) and 18:3 also were evaluated for oxidative stability. Samples included Hardin 91 (10.3% 16:0 and 7.4% 18:3), P9322 (10.6 % 16:0 and 2.6 % 18:3), A91-282036 (26.3 % 16:0 and 9.8 % 18:3), and HPLL (23.2 % 16:0 and 3.5 % 18:3). The fatty acid compositions, peroxide values, and cloud points were measured. Elevating 16:0 and/or lowering 18:3 increased the oxidative

stability of soybean oils. The cloud point test confirmed that soybean oils with elevated 16:0 had greater solidification temperature than oils with normal 16:0, and soybean oils with low 18:3 had greater solidification temperatures than oils with normal 18:3 content, but the differences were quite small even though significant.

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ACKNOWLEDGMENT

I deeply appreciate my major professor, Dr. Pamela J. White, for her patience, friendly help, academic guidance, and financial support. I also wish to thank my committee members: Dr. Walter Fehr, Dr. Earl Hammond, Dr. Lawrence Johnson, and Dr. Carl Tipton for their kindness, guidance, and supervision. I am especially indebted to Dr. Fehr and his research group for growing the soybeans used in my project.

I also should thank my parents who have been encouraging and supporting me since I was a child. I must thank my wife and my daughter. Without their support, love, and understanding, I cannot finish my study.

Last, but not least, my thanks should go to my colleagues who shared both good times and bad times with me.

APPENDIX. DIAGRAM OF DEODORIZATION APPARATUS

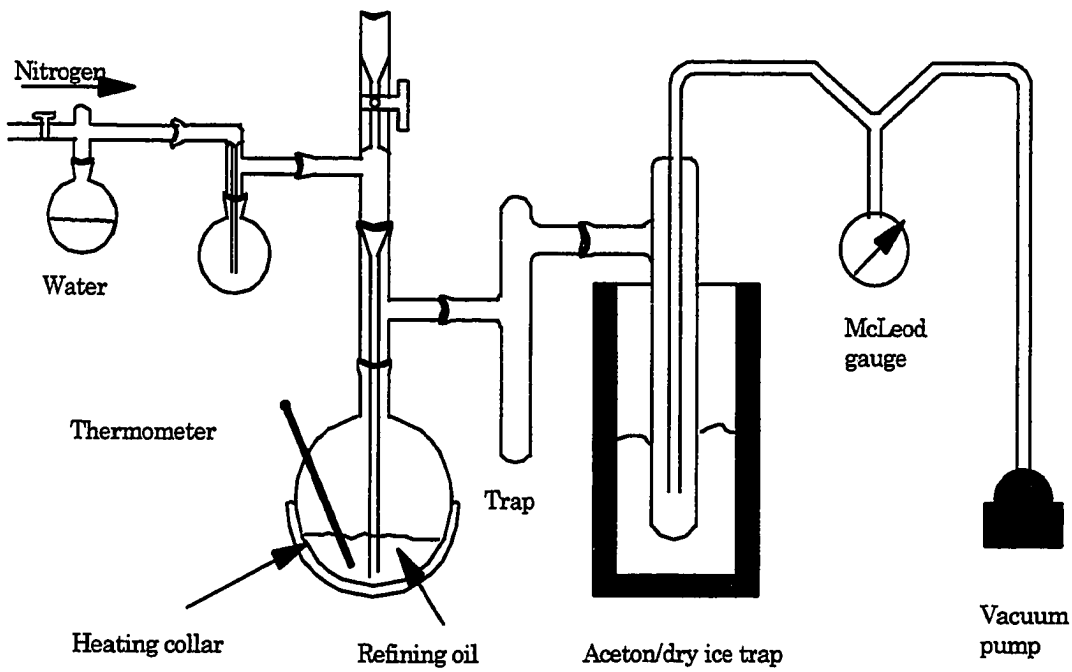

Figure 1. Diagram of deodorization apparatus